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The Effects of Excess Arginine on Lysine Utilization in Rats and Swine

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THE EFFECTS OF EXCESS ARGININE ON LYSINE UTILIZATION IN RATS AND SWINE

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UTILIZATION IN RATS AND SWINE

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

The objective of adding supplemental protein to swine diets is to provide the levels of available essential amino acids and nitrogen necessary for maximum protein synthesis in the pig. Lysine has been determined to be the limiting amino acid in many swine diets (Jones and Pond, 1963; Soldevila and Meade, 1964; Welch et al., 1966; Grimson and Bowland, 1971; Wahlstrom and Libal, 1974). Thus, the real objective of protein supplementation is to provide the amount of lysine necessary for maximum protein synthesis, and if this objective is met the requirements for the other essential amino acids and nitrogen will generally be met.

Since swine diets are formulated to meet the lysine requirement of the pig, it is necessary to derive maximum utilization of the dietary lysine in order to minimize the cost of lean tissue production. Several factors may influence lysine utilization, including the nature of the protein source, amount of heat processing, level of feed intake and relative amount of other amino acids.

Swine diets formulated to meet the lysine requirement of the pig will usually contain an excess of several amino acids including arginine, leucine and phenylalanine plus tyrosine. Currently, there is much debate on the effects of these excess amino acids on pig performance.

Lysine and arginine are both basic amino acids, meaning that they have a positively charged R group when in the physiological pH range. A review of the literature indicates that lysine and arginine share many of the same transport mechanisms throughout the animal's

body. Poultry researchers have demonstrated a very marked effect of excess lysine on arginine transport and metabolism in the chick. It is possible that the excess arginine found in common swine diets may affect lysine utilization and thereby directly affect pig performance.

There are two basic approaches used in the study of the arginine-lysine relationship. The first approach is to alter the arginine content of the diet by reformulation with different sources and levels of protein. This type of research provides little basic knowledge on the arginine-lysine relationship since the arginine levels are confounded by changing protein sources. However, it does provide information on the prospects for improving pig performance by reducing the amount of arginine in common swine diets.

The second approach is to use crystalline arginine to alter the arginine content of the test diets. This is the method used in the present series of experiments. This method allows evaluation of the arginine-lysine relationship with a minimum of confounding but does not provide a method for incorporating the knowledge into practical swine production methods.

The objectives of this study were as follows:

1. To determine the relationship between arginine and lysine in the growing rat.
2. To determine if excess arginine affects the absorption of lysine from the small intestine of the rat.
3. To examine the effects of excess arginine on swine growth, feed intake and feed efficiency.

4. To determine if supplemental lysine could correct any adverse effect of excess arginine in swine.
5. To determine the effect of arginine and lysine supplementation on pig plasma amino acid levels.

REVIEW OF LITERATURE

The terms amino acid imbalance, deficiency, toxicity and antagonism are found throughout the literature in studies involving amino acid and protein nutrition. The use of these terms has been inconsistent and at times inappropriate. It is difficult to make clear distinctions as to their use and meaning since many of the underlying mechanisms involving amino acid relationships have not been elucidated.

An amino acid deficiency occurs when the available dietary level of an essential amino acid is below the amount required by the animal for growth or maintenance, while levels of the other essential amino acids are at or near their respective requirements. In contrast, an imbalance occurs when there is a surplus of essential amino acids other than the one that is growth limiting, with the stipulation that none of the surplus amino acids are present in toxic amounts (Harper et al., 1970). Imbalances and deficiencies may be corrected by supplementing the limiting amino acid to the diet. Specific effects and mechanisms of amino acid imbalances will be discussed in greater detail in a later section.

Amino acid toxicities result from the ingestion of a large excess of a single amino acid (Harper et al., 1970). Symptoms of amino acid toxicities include depressed growth and feed intake (Hill et al., 1945; Kerr and Waisman, 1967) and in some cases the development of pathological lesions (Lillie, 1932; Deb and Biswas, 1965). It is in the area of toxicities that distinctions between various conditions become unclear.

Sauberlich (1961) demonstrated various levels of toxicities for 19 amino acids added individually to the diet of weanling rats fed 6% or 10% casein diets. However, most of the growth depressions could be eliminated by feeding higher levels of protein, suggesting that the effects were due to amino acid imbalances and not toxicities. This is an area where the lack of a physiological basis for observed phenomena make it difficult to explain and classify those phenomena.

An amino acid antagonism refers to the specific effect of one amino acid on the absorption, excretion or metabolism of another amino acid. The arginine-lysine antagonism and the leucine-isoleucine-valine relationship will be discussed in a later section.

Relationships Between Diet and Plasma Amino Acid Levels

Plasma amino acid levels have been used by several researchers to determine the dietary requirement for individual amino acids. Morrison et al. (1961) reported that plasma lysine levels of rats fed graded amounts of lysine remained low and fairly constant at dietary levels of less than .80% but increased in a linear fashion at higher dietary lysine levels. The broken-line response curve for plasma lysine was consistent with the requirement of .80% determined by the growth data. Stockland et al. (1970a) observed that the plasma lysine response curve closely approximated the dietary requirement indicated by rat performance. Peng (1979) used the plasma amino acid response curve to determine the dietary requirement for lysine, histidine, phenylalanine and methionine in the rat.

In work with swine, Mitchell et al. (1968) found nitrogen retention values and plasma amino acid response curves in close agreement when used to estimate the weaned pig's requirement for lysine, isoleucine, leucine and histidine. Bravo et al. (1970) used plasma isoleucine values to determine the isoleucine requirement of the growing pig.

Stockland et al. (1970a) reported the feeding method will alter the shape of the plasma lysine response curve. Plasma values from rats that had undergone a 2-d training period for meal feeding did not exhibit the broken-line response curve characteristic of ad libitum-fed rats. Similar effects of feeding regimen on the plasma lysine response curve were noted by Mitchell et al. (1968) in swine. Data are not available to verify this effect using amino acids other than lysine. The authors of both reports suggest that a period of "metabolic adaptation" of undetermined length must follow changes in the dietary feeding regimen in order for the broken-line response to appear.

Thus it is well documented that plasma amino acid levels provide a method for determining an animal's requirement for individual amino acids. The plasma levels for a given essential amino acid will remain at a low and relatively constant value when dietary levels of that amino acid are below the animal's requirement, due to the large demand for that amino acid for tissue protein synthesis in relation to the amount supplied by the diet. When dietary levels of the amino acid exceed the animal's requirement, the amino acid will accumulate in the plasma.

The plasma amino acid levels of swine have been found to reflect the amount and amino acid composition of the ingested protein (Puchal et al., 1962; Stockland et al., 1970b; Windels et al., 1971; Davey et al., 1973). This relationship is modified by differences in the availability of amino acids from different protein sources (Puchal et al., 1962) and by the growth rate of the animal (Stockland et al., 1970b).

Longnecker and Hause (1959) developed the Plasma Amino Acid Ratio method of determining the limiting amino acid in a protein source for dogs by evaluating changes in postprandial plasma amino acid levels. They determined the relative change in the plasma level of each essential amino acid after a meal and divided that value by the dog's requirement for each amino acid to determine the Plasma Amino Acid Ratio. The amino acid with the smallest positive or largest negative change in its plasma level with respect to the dog's requirement would be the first limiting amino acid. The validity of this method is based on the assumption that amino acids are removed from the plasma at rates proportional to the animal's dietary requirement. Although this assumption has not been disproven in the literature, the Plasma Amino Acid Ratio method has not been widely used.

Plasma amino acid levels in the portal vein are particularly sensitive to the level and availability of amino acids in the diet, since the portal vein levels represent amounts absorbed from the small intestine prior to alterations by liver metabolism and uptake by the body tissues. Guggenheim et al. (1960) observed differences in the portal plasma lysine level of rats fed either unheated, heat-processed

or overheated soybean meal. Rats fed the heat-processed soybean meal had the highest average portal plasma lysine values at 30, 60 and 120 min postfeeding, while rats fed the overheated soybean meal had the lowest values. Similar differences in portal plasma lysine levels were observed by Wheeler and Morgan (1958) in rats fed raw or overheated pork and by Shimada and Zimmerman (1973) in swine fed either raw extracted or heated soybean meal. These differences reflect the well known effects of heating on the availability of lysine from these protein sources. Heat processing soybean meal will improve the availability of lysine due to the destruction of trypsin inhibitors and partial denaturation of the tertiary protein structure. Overheating both pork and soybean meal will reduce lysine availability because the epsilon amino group of lysine is susceptible to being complexed with carbohydrates.

Specific Effects of Amino Acid Imbalances

Early researchers in the area of amino acid imbalance demonstrated that when rats were fed a diet which was supplemented with an amino acid other than the one that was first limiting for growth, the deficiency of the first limiting amino acid appeared to be more severe and the growth rate of the rats was depressed. These early investigations have been reviewed adequately elsewhere (Harper, 1956; Harper et al., 1970) and will not be discussed here.

A severe and very rapid decline in food intake occurs when amino acid imbalanced diets are fed to rats. The food intake depression usually occurs within 6 to 12 h after the imbalance diet is offered (Harper et al., 1970). Other research indicated that when the food

intake of rats fed an imbalanced diet was equalized with the food intake of control rats, the growth rates of the two groups were nearly equal (Kumta and Harper, 1961; Klain et al., 1962; Harper et al., 1966; Harper and Rogers, 1966; Leung et al., 1968). The rats were apparently able to utilize the protein from the imbalanced diets as effectively as the protein from the balanced diets, and the growth depression of rats fed imbalanced diets ad libitum was due mainly to reduced feed intake.

Harper et al. (1964) examined several theories on how an amino acid imbalance could reduce food intake so rapidly. The most consistent observation was that the plasma level of the limiting amino acid dropped markedly after rats were fed a diet that had been supplemented with the second or third limiting amino acid. This was apparently due to increased utilization of the limiting amino acid by the tissues, since an amino acid imbalance has been shown to increase retention of the limiting amino acid (Harper and Rogers, 1965). Thus, the plasma amino acid pattern of rats fed an imbalanced diet resembles the pattern that would be seen if a diet much more deficient in the limiting amino acid had been fed. Furthermore, the researchers noted that rats force-fed a diet which is severely deficient in one amino acid will develop pathological lesions and die. Rats that are given a choice between an imbalanced diet (that would support growth if consumed) and a protein-free diet (that will not support growth) will invariably choose the protein-free diet. Although the protein-free diet does not support growth and the rats will eventually die of protein starvation, it does provide a plasma amino acid pattern similar to the pattern seen in rats fed a balanced diet.

Based on this series of facts, Harper and co-workers (1964) postulated that the food intake depression occurs by the following mechanism: the imbalanced diet creates a plasma amino acid pattern similar to the pattern created by a severely deficient diet. In some way the rats sense the distorted pattern and an appetite-depressing mechanism is triggered to decrease food intake. Harper et al. (1964) noted that this may be a protective mechanism to avoid the harmful effects of a severely deficient diet, although it is a faulty mechanism since imbalanced diets will support growth if food intake is maintained.

Research conducted by Leung and Rogers (1969) provides evidence for the plasma amino acid appetite control mechanism proposed by Harper et al. (1964). Leung and Rogers (1969) found that when small amounts of the limiting amino acid were infused into the carotid artery of rats fed an amino acid imbalanced diet the food intake depression was almost entirely eliminated. When the growth limiting amino acid was infused into the jugular vein of rats fed an imbalanced diet, the food intake depression was not corrected, indicating that the appetite control mechanism was located somewhere in the brain. This study also provides evidence that it is the decrease in the plasma level of the growth limiting amino acid that causes appetite suppression and not the excess of the other plasma amino acids. Similar results were noted by Tobin and Boorman (1979) with infusion studies using cockerels.

Continued research in this area by Leung and Rogers (1970) revealed that electrically induced lesions on the ventromedial hypothalamic nuclei (the traditionally recognized neural satiety center) failed to

prevent food intake depressions caused by amino acid imbalanced diets, indicating that this area was not involved in food intake regulation via the plasma amino acid pattern. Leung and Rogers later found that lesions on certain areas of the prepyriform cortex (Leung and Rogers, 1971; Rogers and Leung, 1973) and on the medial amygdala (Rogers and Leung, 1973) did prevent a food intake depression in rats fed diets low in or devoid of a single essential amino acid.

Thus, it appears there are certain areas of the rat brain that are sensitive to changes in the blood plasma amino acid pattern and that these areas exert an effect on feed intake in an attempt to restore the plasma amino acid values to normal. The decrease in food intake is then the main cause of the depressed growth rate in rats fed amino acid imbalanced diets, while the efficiency of protein utilization is relatively unaffected.

Leucine-Isoleucine-Valine Relationship

Additions of excess leucine to a low protein diet have been found to depress the growth and feed intake of rats (Harper et al., 1954, 1955; Spolter and Harper, 1961) and poultry (D'Mello and Lewis, 1970b,c, 1971; Boldizsar et al., 1973; Tuttle and Balloun, 1976; Smith, 1980). These same studies indicated the effects of leucine were due to changes in isoleucine and valine utilization, since only isoleucine and valine supplementation will correct the growth depression. Animals fed excess leucine also have reduced plasma isoleucine and valine levels (Tannous et al., 1966; D'Mello and Lewis, 1970b, 1971; Tuttle and Balloun, 1976; Shinnick and Harper, 1977; Smith and Austic, 1978).

Research examining changes in branched-chain amino acid enzyme levels has provided some evidence that leucine increases valine and isoleucine catabolism. Leucine, isoleucine and valine first undergo a reversible transamination step which is regulated by branched-chain amino acid aminotransferase. The resulting alpha-keto acid undergoes oxidative decarboxylation mediated by branched-chain amino acid dehydrogenase. Boorman and Buttery (1972) found that 3% leucine had no effect on branched-chain amino acid aminotransferase in chicks, while Smith and Austic (1978) did note an increase in this enzyme when 2.25% leucine was fed. Studies by Wohlhueter and Harper (1970), Khatra et al. (1977) and Shinnick and Harper (1977) indicated that excess leucine can increase the liver branched-chain amino acid dehydrogenase activity of rats.

Phansalker et al. (1970) observed that large doses of leucine increased $^{14}CO_2$ expiration from rats fed L-(U-^{14}C) isoleucine. Boldiszar et al. (1973) did not detect an increase in $^{14}CO_2$ from (U-^{14}C) valine when chicks were fed 4% and 5% leucine diets. A leucine level of 2.25% did increase the $^{14}CO_2$ expiration of chicks fed (1-^{14}C) isoleucine and (1-^{14}C) valine in work by Smith and Austic (1978), but the increases were small and accounted for only 2% of the ingested valine and isoleucine.

Shinnick and Harper (1977) noted that 5% leucine increased the branched-chain amino acid dehydrogenase activity in rats, but they were unable to detect an increase in $^{14}CO_2$ expiration from L-(1-^{14}C) valine. Although an increase in the catabolism of isoleucine and valine may play a role in the leucine-isoleucine-valine antagonism, it does not appear to be the major cause of the deleterious effects.

A leucine-isoleucine-valine relationship has been demonstrated to some extent in swine. Taylor et al. (1977a) found that increasing dietary leucine from 1.30% to 2.00% decreased the gain to feed ratio and percentage lean in ham of growing pigs fed .38% isoleucine but not in pigs fed .45% isoleucine. They also observed a marked increase in pig performance due to isoleucine supplementation of the diet. These results indicate that excess leucine may affect pig performance when isoleucine-deficient diets are fed but not when diets containing adequate isoleucine are fed. Other work by Taylor et al. (1977b) using similar isoleucine levels and either 1.20% or 1.50% leucine revealed no evidence of an isoleucine x leucine interaction.

Plasma isoleucine and valine levels in swine are readily affected by dietary leucine content. Mitchell et al. (1968) and Oestemer (1973) found that increasing levels of dietary leucine caused a linear decrease in plasma isoleucine and valine, but pig performance was not affected. Excess leucine reduced the plasma isoleucine and valine levels of growing pigs in work by Henry et al. (1976), but the daily gain and feed intake of the pigs were unaffected. These researchers concluded that there was no reason to correct the isoleucine and valine requirements of growing pigs for dietary leucine content.

Arginine-Lysine Antagonism in Poultry

Research into the arginine-lysine antagonism in poultry was stimulated by the observation that chicks fed a casein diet have a higher arginine requirement than chicks fed a corn-soybean meal diet. Snyder

et al. (1956) found that chicks fed a corn-soybean meal diet had an arginine requirement of 1.11%, while chicks fed a 22% casein diet had an arginine requirement of 1.73%. Fisher et al. (1960) fed a 20% casein diet, a 15% protein isolated soybean protein diet and the 15% protein isolated soybean protein diet plus an amino acid mixture to give an amino acid composition similar to the 20% casein diet. The amino acid addition to the isolated soybean protein diet depressed chick gains to the level of chicks fed the 20% casein diets, indicating that the amino acid composition of the casein diet was responsible for depressed chick performance. The most notable difference between casein protein and soybean meal protein is the high lysine to arginine ratio of the casein diet. Supplemental arginine corrected the growth depression caused by the 20% casein diet and the isolated soybean protein plus amino acids diet.

Jones (1961) found that a 2% lysine addition to a basal diet containing 18% casein and 10% gelatin decreased chick gains and caused toxicity symptoms such as nervousness and leg tremors. Dean and Scott (1968) added 1% lysine to a balanced amino acid basal diet. The lysine addition depressed daily gain by 33% and feed intake by 16%, and up to .73% additional arginine was required to restore normal performance. Wilburn and Fuller (1975) added .45% lysine to a corn-soybean meal diet to give a total lysine content similar to that of a 25% casein diet. Chicks fed the high lysine diet had reduced gains and demonstrated a more marked response to arginine supplementation when compared to the controls.

Allen and Baker (1972) found that birds receiving a diet containing 1.19% lysine had greater gains and feed intakes than those fed 2.44% lysine. When the low-lysine group was pair-fed to the high lysine group, gains were still lower on the high lysine diet, indicating that excess lysine affected efficiency of feed utilization as well as feed intake.

Work by O'Dell and Savage (1966) indicated a true competitive antagonism between lysine and arginine. They noted that dietary lysine additions depressed the growth of chicks and that this growth depression was corrected by supplemental arginine. When arginine was fed in excess, the lysine requirement of the birds increased. D'Mello and Lewis (1970a) found that only supplemental arginine corrected the growth depression resulting when excess lysine was added to a methionine deficient basal diet, in spite of the fact that methionine, not arginine, was growth limiting in the basal diet. Excess lysine also reduced plasma arginine levels of chicks in this study but did not affect the other plasma amino acid levels.

Considerable research has been conducted to determine the mechanism of the lysine-arginine antagonism in the chick. Kadirvel and Kratzer (1974) concluded that competition for absorption from the small intestine was not involved in the antagonism since lysine had little effect on arginine absorption from segments of chick small intestine. Excess lysine had no effect on trypsin activity or carboxypeptidase B activity in chick studies by Jones et al. (1967).

Nesheim (1968a) observed that chicks fed a basal diet containing 1.6% lysine and 1.8% arginine excreted 1.3 mg of arginine in the urine during the 6 h postfeeding. When 2% L-lysine was added to the basal diet, urinary arginine excretion increased to 4.6 mg during the 6 h postfeeding. This amount represented about 6% of the total arginine intake. Other results from the same series of studies indicated that a 1.25% lysine addition caused 8.4% of the arginine intake to be excreted in the urine, while a 2.00% lysine supplement caused 12.7% of the arginine intake to be excreted.

Boorman et al. (1968) infused 0, .5, 1.0, 2.0 and 4.0 µmol/kg body wt of lysine into the bloodstream of cockerels. Arginine reabsorption from the renal tubules dropped from 96.9% to 66.4%. Thus, competition for reabsorption from the renal tubule and increased urinary arginine excretion is at least partially responsible for the increased arginine requirement of chicks fed high lysine diets.

Nesheim (1968b) noted that chicks genetically selected for a high arginine requirement had increased kidney arginase activity and grew less on a low arginine diet as compared to birds selected for a low arginine requirement. When levels of .5%, 1.0% and 1.5% lysine were added to a soy protein basal diet, kidney arginase activity showed a linear increase in both the high and low arginine requirement strains. Jones et al. (1967) and Austic and Scott (1975) also detected increases in renal arginase activity due to increasing levels of dietary lysine.

A reduction in liver glycine transamidinase activity and reduced creatine synthesis may also be involved in the lysine-arginine

relationship. Jones et al. (1967) observed decreased hepatic glycine transamidinase activity in chicks fed high lysine diets and a 1% creatine supplement partially corrected the growth-depressing effect of excess lysine. Decreased hepatic glycine transamidinase activity in response to lysine supplementation was also observed by Austic and Nesheim (1971). Glycine transamidinase is an enzyme necessary for the synthesis of creatine from arginine and glycine.

A food intake regulating mechanism sensitive to plasma arginine levels may also be operating in the lysine-arginine antagonism in poultry. Jones et al. (1967) detected a decrease in plasma arginine levels within 6 h of feeding a high lysine diet to chicks, while renal arginase activity did not increase until 48 h postfeeding. The exact cause of the early decrease in plasma arginine is not known, but it may precipitate a decline in food intake. A depression in food intake within 12 h of feeding a high lysine diet to chicks was noted by Austic and Scott (1975).

The effects of lysine on arginine utilization in chicks appears to occur via several mechanisms, including increased urinary arginine excretion, increased renal arginase activity and depressed hepatic glycine transamidinase activity. Increased urinary arginine excretion and renal arginase activity would reduce the amount of arginine available to the bird for protein synthesis. The depressed hepatic glycine transamidinase activity may create a deficiency of creatine in the bird. Food intake regulation via the plasma amino acid pattern, similar to the effects of an amino acid imbalance previously noted, may

also play a role in the overall depression in bird performance. The relative importance of each mechanism has not been determined.

Arginine-Lysine Antagonism in Mammals

Although the mechanism of the arginine-lysine antagonism in poultry has been worked out in great detail, relatively little work has been done to establish the existence and determine the mechanism of this relationship in mammals. Some work has been conducted dealing with competition for intestinal absorption and renal reabsorption, but virtually no work has been done to determine possible changes in enzyme activity or to determine the role of food intake and protein utilization in the antagonism. Possible mechanisms for the arginine-lysine antagonism, as well as the relative importance of each mechanism, have not been adequately researched.

Finch and Hird (1960) used Michaelis-Menten kinetics to predict the relative uptake of various amino acids from isolated segments of rat small intestine. They were able to predict the order of affinity for uptake for most amino acids with the notable exceptions of lysine, arginine and ornithine. The rates of uptake for these amino acids were not predictable from their Michaelis-Menten constants, and the authors suggested that the basic amino acids were not transported by the same mechanism as the other amino acids.

McCarthy et al. (1964) studied amino acid uptake by duodenal segments taken from human patients with cystinuria, a genetic disease characterized by excessive amounts of cystine in the urine. Uptake of L-lysine, L-arginine, DL-ornithine and L-cystine was low in cystinuric

patients compared to noncystinuric controls. DL-cystine, DL-leucine and L-phenylalanine were taken up equally by both groups. These results suggest a separate transport mechanism for the basic amino acids and cystine, since subjects without the genetic ability to absorb cystine also had reduced capacity for lysine, arginine and ornithine absorption.

Adibi et al. (1967) infused various mixtures of amino acids into the small intestine of conscious human subjects. When an 8 mM solution of lysine was infused, 60% of the lysine was absorbed. Lysine absorption dropped to 43% when arginine was added to the infusion solution in an equimolar amount.

Buraczewski et al. (1970) provided direct evidence of competition between lysine and arginine for intestinal absorption in swine. A pig was fitted with re-entrant cannulas at the distal duodenum and proximal ileum so that absorption from the jejunum could be measured. When the arginine to lysine weight ratio of the infusion solution was .80 to 1, 93% of the lysine and 96% of the arginine was absorbed. A tenfold increase in the lysine concentration of the infusion solution decreased arginine absorption to 52%, while lysine absorption remained high. Similarly, when the arginine concentration was increased tenfold, lysine absorption decreased to 28%.

These results indicate that lysine and arginine compete as free amino acids for absorption from the small intestine. It appears that arginine competes for absorption more successfully than lysine, since arginine depressed lysine absorption much more than lysine depressed arginine absorption. The latter result agreed with data from Orten

(1968), who found that arginine was absorbed much more rapidly than lysine in the human small intestine.

The work of Buraczewski et al. (1970) illustrated the physiologic relationship between lysine and arginine, but it may have limited application in normal swine digestion. Arginine to lysine ratios of .08:1 and 7.9:1 were used, which are well outside of the range found in swine diets with natural protein sources. Also, the placement of the cannulas allowed for absorption from approximately one-third of the small intestine, meaning that the absorptive capacity had been reduced by two-thirds. This combination of extreme arginine to lysine ratios and reduced intestinal capacity may have contributed to their extreme results.

Alimon and Farrell (1980) compared the ileal digestibility of lysine from fish meal, meat meal, peanut meal and soybean meal. Peanut meal, which had the highest arginine to lysine ratio (3.0:1), also had the highest lysine digestibility (83%). Fish meal had the lowest arginine to lysine ratio (.8:1) but also the lowest lysine digestibility (75%). The lysine digestibility of these sources was not adversely affected by their arginine content. Other factors of protein quality, such as type of processing, may play a greater role in affecting lysine digestibility.

It remains to be demonstrated that competition between amino acids for intestinal absorption occurs in swine fed natural diets. Arginine to lysine ratios of swine diets are between .5:1 and 2.0:1. Also, digestion and absorption of amino acids occurs along the entire length of

the small intestine (Alimon and Farrell, 1980). In pigs fed a diet with a high arginine to lysine ratio, arginine may compete and be absorbed preferentially over lysine in the upper portions of the small intestine, but as this process continues the arginine to lysine ratio of the digesta would drop and lysine absorption would increase as the digesta moves down the small intestine. Thus, lysine absorption would be delayed, but overall lysine digestibility may not be affected.

Lysine and arginine also compete for reabsorption from the renal tubules. Renal transport of the basic amino acids is less efficient than for the other amino acids. Beyer et al. (1946) could not raise plasma tryptophan, leucine, iosleucine and valine levels of dogs high enough to exceed the renal reabsorptive capacity for those amino acids. Wright et al. (1947) found that filtered histidine, methionine, leucine, valine and tryptophan were readily reabsorbed from the renal tubules in dogs, and plasma values high enough to cause urinary excretion could not be attained without causing nausea in the dog. However, this same study indicated that lysine began to appear in the urine when plasma lysine levels reached 22 mg/100 ml. Urinary arginine excretion began at plasma arginine levels of 33.8 mg/100 ml.

Kamin and Handler (1951) demonstrated the effects of various amino acids on arginine reabsorption in the canine kidney. Control dogs excreted 3.8 µg/min of arginine in the urine, while dogs infused with lysine excreted 91 µg/min of arginine. Histidine infusion increased arginine excretion to 38 µg/min.

Webber et al. (1961) used female dogs to study interactions of amino acids in renal tubular transport. Increasing levels of lysine infusion decreased renal arginine reabsorption from 99.6% to 3.3%. Interestingly, plasma arginine levels doubled during the lysine infusions. This latter result cannot be rationalized on any known physiological basis. Increasing levels of arginine infusion decreased renal lysine reabsorption from 99.5% to -27.3%. The arginine infusions nearly doubled plasma lysine levels.

Webber et al. (1961) noted that histidine had a less marked effect on arginine and lysine reabsorption. Cystine reabsorption was drastically reduced by both arginine and lysine infusions, while methionine reabsorption was decreased by arginine infusion only. The relationship between lysine, arginine and cystine found by Webber et al. (1961) correlates with the work of McCarthy et al. (1964), who noted a defective intestinal uptake of these amino acids in human patients with cystinuria.

These studies indicated a competitive antagonism among the basic amino acids, and possibly the sulfur-containing amino acids, for reabsorption from the renal tubules. The studies were conducted with dogs and their applicability to swine is unknown. Also, very extreme plasma amino acid levels were used to demonstrate the relationships. Plasma levels of arginine and lysine during the infusion studies of Webber et al. (1961) were too high to measure.

Southern and Baker (1982) examined the effects of arginine on weaned pig performance and urinary amino acid excretion. The diets

contained 1.0% lysine and either .8%, 1.3%, 1.8%, 2.3% or 2.8% arginine. Daily gains for the five treatment groups were 407, 407, 397, 347 and 342 g/d, respectively. Daily feed intake followed a similar pattern and gain/feed was not affected.

At the end of the 26-d growth trial, two pigs from each treatment were placed in metabolism cages and a 24-h collection of urine was made. Urinary lysine excretions for the five treatment groups were 94, 480, 381, 144 and 1638 μmol/24 h, respectively. Thus, urinary lysine excretion was increased when dietary arginine was increased from 2.3% to 2.8%. The increased loss of lysine in the urine was not reflected in pig performance, since daily gains of pigs fed 2.3% or 2.8% arginine during the growth trial were not significantly different. Increased loss of lysine in the urine caused by excess dietary arginine was not a factor in pig performance in this experiment.

There are reports in the literature dealing with the effects of feeding excess arginine to swine. Some researchers have varied the arginine to lysine ratio of the experimental diets by varying the source and level of protein in the diet. These types of data are confounded by changing protein sources and levels of available essential amino acids, but they do provide insight on the potential for improving pig performance by lowering the arginine content of the diet via reformulation.

Wahlstrom et al. (1981) fed 1.15% lysine and either 1.44%, 1.13% or .91% arginine to weaned pigs weighing 8.1 kg initially. Arginine content of the diets was varied by removing soybean meal and adding synthetic lysine. This method reduced the crude protein content of the

diets from 20.6% to 14.4% and the tryptophan content from .24% to .15%. The National Research Council (NRC, 1979) lists the tryptophan requirement of the 5- to 10-kg pig as .15%. The .91% arginine diet also contained 10% whey.

Pigs fed 1.13% arginine tended to have higher daily gains than the other two groups, but there were no significant differences. The lack of a response on the low arginine diet may be due to the low tryptophan content of that diet. This possibility illustrates the difficulty in drawing conclusions from data confounded by changing protein sources and levels.

A similar study was conducted by Anderson and Lewis (1982) in which they lowered the arginine content of a growing-finishing diet by changing protein source and level. The finishing diets contained .58% lysine and either .88%, .61%, .56% or .34% arginine. The pigs showed a positive response in daily gain to the first two reductions in dietary arginine content, but pigs fed the .34% arginine diet had gains similar to pigs fed .88% arginine. Feed intake and feed efficiency followed a similar pattern but were not significantly different. Again, the low tryptophan and(or) crude protein content of the .34% arginine diet may be responsible for the lack of response in pigs fed that diet.

Miller et al. (1981) used growth trials and nitrogen balance studies to examine the effects of an improved amino acid balance on pig performance. They compared a corn-soybean meal diet (arginine to lysine ratio, 1.20:1; leucine to isoleucine ratio, 2.83:1) with an improved amino acid balance diet containing wheat, soybean meal,

blood meal and whey (arginine to lysine ratio, 1.00:1; leucine to isoleucine ratio, 2.50:1).

Pig performance tended to be improved by the balanced amino acid diet in the first growth trial, but there was no trend in the second trial. Their overall conclusion was that pig performance was unaffected by amino acid balance.

Nitrogen balance studies were conducted with four weight groups of pigs (5 to 10 kg, 10 to 20 kg, 20 to 35 kg and 35 to 60 kg). Although there was only one significant difference, in almost all cases the values for percentage apparent N digestibility, percentage N retention and apparent biological value were slightly higher for pigs fed the corn-soybean meal diet. The corn-soybean meal diet gave a significantly higher percentage N retention and apparent biological value in the 20 to 35-kg pigs. Improving the amino acid balance of swine diets by lowering the arginine and leucine contents gave little advantage in either pig performance or nitrogen balance in this study.

Easter and Baker (1977) conducted a study to determine if arginine had an antibiotic effect in weaned pigs. Their data are useful in illustrating the effect of arginine on pigs fed various levels of lysine and crude protein. Four levels of added arginine (0%, .50%, 1.00% and 1.50%) were used in combination with protein levels of 12%, 19% and 26%. Increasing levels of arginine caused a linear decrease in daily gain, daily feed intake and gain to feed ratio of pigs fed the 12% protein basal diet. Arginine did not affect the performance of pigs fed the 19% or 26% protein diets. The effect of arginine depends on the

level of crude protein (or lysine) in the diet as well as the arginine to lysine ratio. It appears that the pigs must be in the linear portion of their protein (lysine) growth response curve before arginine will affect pig performance.

Tanksley (1981) reported the results of a study conducted to evaluate the effects of excess arginine on pig performance. Additions of .3% and .6% L-arginine hydrochloride to a basal diet containing sorghum, soybean meal and blood meal had no effect on the performance of pigs from 10 to 21 kg.

Southern and Baker (1982) used a 19% protein corn-soybean meal-whey-fish meal basal diet to study the effects of arginine on weaned pig performance. Arginine additions of 0%, .67%, 1.33% and 2.00% caused a linear decrease in weight gain and feed consumption but had no effect on feed efficiency. Plasma lysine and histidine were decreased linearly. In two subsequent trials, a 2% arginine supplement decreased pig gains and feed intake but had no effect on feed efficiency. Plasma lysine and histidine were reduced by supplemental dietary arginine. A .5% lysine supplement to the diet failed to increase pig performance and also failed to correct the adverse effects of arginine on pig performance. The authors concluded that excess arginine caused an amino acid imbalance rather than an arginine-lysine antagonism.

MATERIALS AND METHODS

Experiments With Rats

A 9% casein basal diet supplemented with threonine, cystine and isoleucine was fed in all rat experiments. The basal diet met the requirements of the growing rat as established by Rama Rao et al. (1959) for all essential amino acids except lysine and arginine. In Exp. A and B, arginine was also supplemented to the basal diet to meet the rat's dietary requirement of .41%. In Exp. C, the arginine content of the basal diet was .35%. The lysine content of the basal diet was .64%, which is below the requirement of .90% proposed by Rama Rao et al. (1959) but adequate for the lysine requirement of .60% indicated by Stockland and Meade (1970) and Stockland et al. (1970a). A comparison of the amino acid composition of the basal diet with the dietary requirements of the rat as indicated by Rama Rao et al. (1959) and Stockland and Meade (1970) is presented in table 1.

The rats were housed individually in screen-bottomed cages and treatments were randomly allotted within each level of the cage rack. Water was supplied ad libitum and light was provided for 9 h each day. Room temperature was 21 C.

At the end of each experiment, the rats were anesthetized with ether and a 4- to 5-ml blood sample was taken via heart puncture using a syringe and a 20 gauge, 3.8-cm needle. Each sample was then transferred to a centrifuge tube. Sodium heparin was used as the anticoagulant in the syringes and centrifuge tubes.

TABLE 1. COMPARISON OF THE RAT BASAL DIET COMPOSITION AND THE AMINO ACID REQUIREMENT OF THE GROWING RAT

Amino acid	Content of basal diet[a]	Requirement[b]	Requirement[c]
	%	%	%
Lysine	.64[d]	.90	.60
Arginine	.41[d]	--	.41
Histidine	.25	.21	.21
Threonine	.51	.51	.51
Valine	.58	.56	.56
Methionine + cystine	.49	.50	.45
Isoleucine	.55	.55	.55
Leucine	.83	.69	.69
Phenylalanine + tyrosine	.93	.72	.66
Tryptophan	.12	.11	.11

[a] Basal diet contained 9% casein supplemented with threonine, cystine and isoleucine.
[b] Requirement indicated by Rama Rao et al. (1959).
[c] Requirement indicated by Stockland and Meade (1970).
[d] Arginine content of basal diet for Exp. A and B. Basal diet of Exp. C contained .35% arginine.

Experiment A. Forty-eight male Holtzman rats were fed the basal diet (treatment 1, table 2) ad libitum for a 2-d adjustment period. The rats were then weighed without fasting and allotted on a weight basis to six treatments for a total of eight rats per treatment. The average beginning weight of the rats was 76.5 g.

The composition of the diets for Exp. A is shown in table 2. Treatments were created by adding L-arginine hydrochloride and(or) L-lysine hydrochloride to the basal diet in place of dextrose. Diets were made isonitrogenous by substituting glutamic acid for dextrose as needed. The six treatments consisted of two lysine levels (.64% and .92%) and three arginine levels (.41%, 1.13% and 2.39%) in a 2 x 3 factorial arrangement. Because of the previously indicated difference

TABLE 2. PERCENTAGE COMPOSITION OF RAT DIETS FOR EXPERIMENTS A AND B

Ingredient	Treatment no. 1	2	3	4	5	6
Casein	9.00	9.00	9.00	9.00	9.00	9.00
Corn oil	5.00	5.00	5.00	5.00	5.00	5.00
Vitamin mix[a]	2.20	2.20	2.20	2.20	2.20	2.20
Salt mix[b]	4.00	4.00	4.00	4.00	4.00	4.00
Dextrose	73.48	74.57	76.20	73.64	74.73	76.36
DL-threonine[c]	.26	.26	.26	.26	.26	.26
L-cystine	.22	.22	.22	.22	.22	.22
L-isoleucine	.09	.09	.09	.09	.09	.09
L-arginine·HCl	.11	1.10	2.59	.11	1.10	2.59
L-lysine·HCl	--	--	--	.28	.28	.28
L-glutamic acid	5.64	3.56	.44	5.20	3.12	--
Chemical analysis						
Lysine	.64	.64	.64	.91	.90	.95
Arginine	.41	1.12	2.39	.41	1.14	2.39

[a] Provided the following per kg of diet: vitamin A, 19800 IU; vitamin D, 2200 IU; alpha-tocopherol, 110 mg; ascorbic acid, 990 mg; inositol, 110 mg; choline chloride, 1650 mg; menadione, 49.5 mg; p-aminobenzoic acid, 110 mg; niacin, 99 mg; riboflavin, 22 mg; pyridoxine·HCl, 22 mg; thiamine·HCl, 22 mg; calcium pantothenate, 66 mg; biotin, 440 mcg; folic acid, 1980 mcg; and vitamin B_{12}, 29.7 mcg.

[b] Provided the following per kg of diet: calcium carbonate, 10.75 g; calcium phosphate·H_2O, 3403 mg; cobalt chloride, 2 mg; copper sulfate, 10.72 mg; dipotassium diphosphate, 15.14 g; ferric citrate, 985.2 mg; magnesium sulfate, 3655 mg; manganese sulfate, 12.52 mg; potassium iodide, 28.64 mg; sodium chloride, 6.00 g; and zinc chloride, 8.92 mg.

[c] DL-threonine was considered to be 50% available.

in the estimated lysine requirement of the growing rat, two levels of lysine were used in this experiment and in Exp. C. The lower lysine level was considered to be marginally adequate or deficient, while the higher level was considered definitely adequate.

The experimental diets were fed ad libitum for 24 d, and the rats were weighed without fasting on d 7, 14, 21 and 24. Feed intake was recorded daily for the first 3 d and less frequently thereafter in order to determine early changes in feed intake. Blood samples were obtained without fasting at the end of the experiment. The plasma samples were pooled randomly in pairs within treatments to form four pooled samples per treatment which were analyzed without duplication.

Experiment B. Fifteen male Holtzman rats, weighing 131 g initially, were fed the basal diet (treatment 1, table 2) ad libitum for a 5-d adjustment period. At 1700 h on the fifth day, the feed cups were removed and the rats were weighed, allotted to three treatments on the basis of weight and then fasted overnight until 0600 h the next day. At that time, the rats were fed their respective experimental diets for a 2-h period. Four hours after the end of the feeding period, blood samples were obtained in the manner previously described. The blood samples from this experiment were analyzed individually without duplication.

The three treatments used in Exp. B contained .64% lysine and either .41%, 1.13% or 2.39% arginine. They were identical in composition to treatments 1, 2 and 3, respectively, from Exp. A (table 2).

Experiment C. The composition of the diets used in Exp. C is listed in table 3. The six treatments consisted of two lysine levels (.66% and .86%) and three arginine levels (averaging .35%, 2.87% and 5.12%) in a 2 x 3 factorial arrangement. Treatments were created by adding L-lysine hydrochloride and(or) L-arginine hydrochloride at the expense of dextrose. The diets were not isonitrogenous.

The rats used in Exp. C were the same male Holtzman rats used in Exp. A. Since blood was taken from these rats for analysis in Exp. A, the rats were allowed to recover for 20 d on a stock laboratory rodent diet fed ad libitum. The rats weighed an average of 314 g at the end of the recovery period.

Since the feed intake of rats during the single 2-h feeding period in Exp. B was rather low, an attempt was made in Exp. C to increase consumption by adapting the rats to a meal feeding regimen. The rats were fed the stock diets in single 2-h feeding periods daily for 13 d following the recovery period. During this adaptation period, the rats lost an average of 21.6 g of body weight, indicating they were unable to increase their consumption enough during the 2-h feeding periods to maintain body weight.

At the end of the adaptation period, 36 rats with an average weight of 292.4 g were selected and allotted on a weight basis to the six dietary treatments for a total of six rats per treatment. The rats were fed daily in single 2-h feeding periods for 4 d. Blood samples were collected 4 h after the end of the feeding period on d 4. The plasma samples were pooled randomly in pairs within treatments to form

TABLE 3. PERCENTAGE COMPOSITION OF RAT DIETS FOR EXPERIMENT C

Ingredient	Treatment no. 1	2	3	4	5	6
Casein	9.00	9.00	9.00	9.00	9.00	9.00
Corn oil	5.00	5.00	5.00	5.00	5.00	5.00
Vitamin mix[a]	2.20	2.20	2.20	2.20	2.20	2.20
Salt mix[b]	4.00	4.00	4.00	4.00	4.00	4.00
Dextrose	79.14	76.36	73.57	78.86	76.08	73.29
DL-threonine[c]	.26	.26	.26	.26	.26	.26
L-cystine	.22	.22	.22	.22	.22	.22
DL-isoleucine[c]	.18	.18	.18	.18	.18	.18
L-arginine·HCl	--	2.78	5.57	--	2.78	5.57
L-lysine·HCl	--	--	--	.28	.28	.28
Chemical analysis						
Lysine	.65	.65	.67	.86	1.03	.83
Arginine	.38	2.57	5.09	.31	3.18	5.16

[a] Provided the following per kg of diet: vitamin A, 19800 IU; vitamin D, 2200 IU; alpha-tocopherol, 110 mg; ascorbic acid, 990 mg; inositol, 110 mg; choline chloride, 1650 mg; menadione, 49.5 mg; p-aminobenzoic acid, 110 mg; niacin, 99 mg; riboflavin, 22 mg; pyridoxine·HCl, 22 mg; thiamine·HCl, 22 mg; calcium pantothenate, 66 mg; biotin, 440 mcg; folic acid, 1980 mcg; and vitamin B_{12}, 29.7 mcg.

[b] Provided the following per kg of diet: calcium carbonate, 10.75 g; calcium phosphate·H_2O, 3403 mg; cobalt chloride, 2 mg; copper sulfate, 10.72 mg; dipotassium diphosphate, 15 14 g; ferric citrate, 985.2 mg; magnsium sulfate, 3655 mg; manganese sulfate, 12.52 mg; potassium iodide, 28.64 mg; sodium chloride, 6.00 g; and zinc chloride, 8.92 mg.

[c] The DL form of these amino acids was considered to be 50% available.

three pooled samples per treatment. The pooled samples were analyzed without duplication.

Statistical Analysis of Rat Experiments. Data from the three rat experiments were analyzed as completely random designs and orthogonal contrasts were used to compare treatment means. In Exp. B, the linear and quadratic effects of arginine levels were examined using the variation among rats within treatments as the error term.

In Exp. A and C, the treatments were in a 2 x 3 factorial arrangement. The contrasts examined the linear and quadratic effects of arginine levels within each lysine level and the lysine main effect. Thus, these experiments were analyzed as nested designs with arginine levels nested within levels of lysine. This did not affect the calculation of the contrasts as the variation among rats within treatments was still the proper error term.

The analyses of variance for all variables in the rat experiments are presented in appendix tables 1 to 10.

Experiments With Swine

The two experiments with swine were conducted in an environmentally controlled nursery room with pens having plastic or plastic-coated expanded metal floors. Room temperature was 27 C during the first 3 wk of each trial and was gradually dropped to 23 C thereafter. Heat lamps provided additional heat for the first 5 d postweaning for the replicates with lighter initial weights. Feed and water were supplied ad libitum and light was provided for 9 h each day. Pig weights and feed intake were recorded weekly during both experiments.

Blood samples were collected from the anterior vena cava of the pigs using a heparinized syringe and an 18 or 20 gauge, 3.8-cm needle. The samples were immediately transferred to centrifuge tubes. Sodium heparin was used as the anticoagulant.

Experiment 1. Ninety-six crossbred weaned pigs averaging 8.1 kg initially were allotted on the basis of weight and ancestry to one of six dietary treatments. There were four pigs per replication and four replications per treatment in the randomized complete block design. Neo-terramycin was administered orally to several pigs that exhibited scour symptoms during the first week postweaning. The symptoms disappeared after 1 or 2 d of treatment.

Blood samples were taken at the end of the 28-d experiment without fasting. The plasma samples were pooled by pen to form four samples per treatment. Two duplicate analyses were conducted on each pooled sample and an average of the two values was used in the statistical analysis. If the two duplicate values did not fall within 5% of their mean, a third analysis was conducted and an average of the three values was used.

Oats groats, corn, fish meal, dried skim milk and supplemental lysine were used to formulate the basal diet containing .94% arginine and 1.03% lysine. The basal diet met all of the amino acid requirements of the 5- to 10-kg pig as established by the National Research Council (NRC, 1979). This comparison is shown in table 4.

TABLE 4. COMPARISON OF THE AMINO ACID CONTENT OF THE BASAL DIETS FOR EXPERIMENTS 1 AND 2 WITH THE AMINO ACID REQUIREMENT OF WEANED PIGS

Amino acid	Basal diet		NRC requirement[b]
	Experiment 1	Experiment 2[a]	
	%	%	%
Lysine	1.03[c]	.92[c]	.95
Arginine	.94[c]	.72[c]	.25
Histidine	.35	.36	.23
Isoleucine	.70	.83	.63
Leucine	1.37	1.62	.75
Methionine + cystine[d]	.60	.64	.56
Phenylalanine + tyrosine[e]	1.20	1.21	.88
Threonine	.63	.69	.56
Tryptophan	.17	.20	.15
Valine	.82	.86	.63

[a] Basal diet of experiment 2 was supplemented with DL-tryptophan.
[b] National Research Council (1979) requirement for 5- to 10-kg pigs.
[c] Based on chemical analysis of the diet; values for all other amino acids are calculated from a feed ingredient composition table (National Research Council, 1979).
[d] Cystine can meet half the requirement for methionine.
[e] Tyrosine can meet half the requirement for phenylalanine.

The composition of the experimental diets is presented in table 5. Treatments were created by adding L-lysine hydrochloride and(or) L-arginine hydrochloride to the basal diet in place of corn. Lysine levels averaging 1.03% and 1.25% and arginine levels averaging .94%, 1.29% and 1.63% were used in a 2 x 3 factorial arrangement. These levels provided arginine to lysine ratios ranging from .75:1 up to 1.58:1.

Data from Exp. 1 were analyzed as a randomized complete block design using a least-squares procedure (Barr et al., 1979). Orthogonal contrasts were used to examine the linear and quadratic effects of arginine within each level of lysine and the lysine main effect. Thus, the factorial treatment arrangement was analyzed as a nested design with levels of arginine nested within levels of lysine. The replication x arginine within lysine mean square, which contained the replication x arginine and the replication x arginine x lysine mean squares, was used as the denominator in the significance tests for the linear and quadratic effects of arginine within lysine levels. The replication x lysine mean square was used to test for the lysine main effect.

Analyses of variance for all variables in Exp. 1 are presented in appendix tables 11 to 13.

Experiment 2. Eighty crossbred weaned pigs weighing 8.0 kg initially were allotted on the basis of weight and ancestry to four dietary treatments. There were four pigs per replication and five replications per treatment for a total of 20 pigs per treatment. Blood samples were collected without fasting at the end of the 26-d experiment

TABLE 5. PERCENTAGE COMPOSITION OF DIETS FOR EXPERIMENT 1

Ingredient	Treatment no. 1	2	3	4	5	6
Yellow corn	34.92	34.55	34.18	34.60	34.23	33.86
Oats groats	48.87	48.87	48.87	48.87	48.87	48.87
Menhaden fish meal	3.50	3.50	3.50	3.50	3.50	3.50
Dried skim milk	10.00	10.00	10.00	10.00	10.00	10.00
Dicalcium phosphate	1.03	1.03	1.03	1.03	1.03	1.03
Limestone	.54	.54	.54	.54	.54	.54
Vitamin premix[a]	.44	.44	.44	.44	.44	.44
Trace mineral salt	.50	.50	.50	.50	.50	.50
L-lysine·HCl	.197	.197	.197	.516	.516	.516
L-arginine·HCl	--	.370	.741	--	.370	.741
Chemical analysis						
Lysine	1.05	1.03	1.00	1.32	1.18	1.29
Arginine	.94	1.34	1.63	.94	1.24	1.62

[a] Provided the following per kg of diet: vitamin A, 4400 IU; vitamin D, 440 IU; vitamin E, 8.8 IU; vitamin K, 3.52 mg; riboflavin, 4.4 mg; pantothenic acid, 17.6 mg; niacin, 28.2 mg; choline, 176 mg; vitamin B_{12}, 17.6 mcg; selenium, 152.4 mcg; penicillin, 55 mg; aureomycin, 110 mg; sulfamethazine, 110 mg; and zinc, 88 mg.

and plasma samples were pooled in pairs within pens to form 10 pooled samples per treatment.

A comparison of the basal diet amino acid content for Exp. 2 with the amino acid requirement of the weaned pig is shown in table 4. Dried skim milk, fish meal and corn were used to formulate the basal diet containing .72% arginine. Tryptophan was added to the basal diet to ensure that it was not growth-limiting. L-lysine hydrochloride or L-arginine hydrochloride were added to create four treatments: three treatments with .92% lysine and either .72%, 1.10% or 1.61% arginine and a positive lysine control (1.10% lysine, .70% arginine). The arginine to lysine ratios of the diets used in Exp. 2 ranged from .64:1 to 1.75:1. The composition of the diets for Exp. 2 is shown in table 6.

Data from Exp. 2 were analyzed as a randomized complete block design using a least-squares procedure (Barr et al., 1979). Orthogonal contrasts were used to examine the linear and quadratic effects of arginine levels at .92% lysine and the overall effect of lysine supplementation (treatment 4 vs treatments 1, 2 and 3; table 6).

Analyses of variance for all variables in Exp. 2 are presented in appendix tables 14 to 19.

Blood Sample Preparation and Analysis

All blood samples from both the rat and swine experiments were prepared and analyzed in an identical manner. Plasma was obtained from each blood sample by centrifugation at 2500 x g for 10 min. At this

TABLE 6. PERCENTAGE COMPOSITION OF DIETS FOR EXPERIMENT 2

Ingredient	Treatment no. 1	2	3	4
Yellow corn	74.51	74.02	73.52	74.26
Menhaden fish meal	3.50	3.50	3.50	3.50
Dried skim milk	19.56	19.56	19.56	19.56
Dicalcium phosphate	1.03	1.03	1.03	1.03
Limestone	.40	.40	.40	.40
Vitamin premix[a]	.44	.44	.44	.44
Trace mineral salt	.50	.50	.50	.50
DL-tryptophan[b]	.06	.06	.06	.06
L-lysine·HCl	--	--	--	.25
L-arginine·HCl	--	.49	.99	--
Chemical analysis				
Lysine	.93	.90	.94	1.10
Arginine	.72	1.10	1.61	.70

[a] Provided the following per kg of diet: vitamin A, 4400 IU; vitamin D, 440 IU; vitamin E, 8.8 IU; vitamin K, 3.52 mg; riboflavin, 4.4 mg pantothenic acid, 17.6 mg; niacin, 28.2 mg; choline, 176 mg; vitamin B_{12}, 17.6 mg; selenium, 152.4 mcg; penicillin, 55 mg; aureomycin, 110 mg; sulfamethazine, 110 mg; and zinc, 88 mg.

[b] DL-tryptophan was considered to be 50% available.

point, any pooling of plasma samples as indicated in the previous methods was performed.

The plasma samples were deproteinized according to the method of Mondino et al. (1971). Plasma proteins were precipitated by adding 2 ml of plasma to 8 ml of a 3.75% sulfosalicylic acid solution (pH 1.8, .3 N lithium citrate). The precipitated proteins were removed by centrifugation at 10,000 x g for 10 min at 0 C. Deproteinized plasma samples were stored in glass vials at -15 C until analysis.

Separation and quantitation of the plasma amino acids were conducted by ion exchange chromatography using a Beckman Model 118BL Amino Acid Analyzer. Concentrations of amino acids are expressed as mg of amino acid per 100 ml of nondeproteinized plasma.

RESULTS

Experiment A

Experiment A was conducted to determine the long-term effects of feeding excess arginine to growing rats. The rats weighed 76.5 g initially and were in the linear portion of their growth curve, although weight gains appeared to be decreasing near the end of the 24-d experiment.

The effects of arginine on growth, feed intake and feed efficiency of rats in Exp. A are presented in table 7. In rats fed .64% lysine, increasing levels of dietary arginine tended to increase daily gains quadratically ($P<.10$) during d 15 to 24, but gains from d 0 to 24 were not affected. Increasing dietary arginine caused a significant linear increase in daily gains of rats fed .92% lysine for all periods except d 15 to 24. A similar but nonsignificant trend in daily gain was apparent during d 15 to 24. Overall, rats fed the high lysine, low arginine diet had the lowest average daily gain and supplemental arginine caused increased growth only in rats fed the high lysine diets.

Average daily feed intake of rats in Exp. A followed a pattern similar to that of daily gain. Feed intake was unaffected by dietary arginine level in rats fed .64% lysine. Rats fed the high lysine, low arginine diet had the lowest feed intake and supplemental arginine increased ($P<.01$) the feed intake of rats fed the high lysine diets. These effects were apparent throughout the 24-d trial.

TABLE 7. EFFECT OF ARGININE ON GROWTH, FEED INTAKE AND FEED EFFICIENCY OF RATS FED AD LIBITUM FOR 24 DAYS (EXPERIMENT A)

Lysine, %	.64			.92		
Arginine, %	.41	1.12	2.39	.41	1.14	2.39
Arginine/lysine	.64	1.75	3.37	.45	1.24	2.60
Treatment no.	1	2	3	4	5	6
			Average daily gain, g			
Days 0 to 7[d]	5.9	6.2	5.9	5.4	6.0	6.4
Days 8 to 14[c]	5.5	5.5	5.4	4.8	5.2	5.6
Days 15 to 24[a]	4.3	5.1	4.7	4.4	5.1	4.9
Days 0 to 24[d]	5.1	5.5	5.3	4.8	5.4	5.5
			Average daily feed intake, g			
Day 1	13.0	13.1	12.4	12.7	13.1	12.7
Day 2[c]	11.3	11.9	11.9	11.5	12.3	12.7
Day 3	12.2	12.6	12.3	12.5	12.4	12.6
Days 4 to 5[d]	14.6	14.6	14.2	13.1	14.6	14.9
Days 6 to 7[c]	14.5	14.4	14.8	14.5	14.3	16.1
Days 8 to 14[d]	16.4	16.4	16.7	15.1	16.3	18.7
Days 15 to 21[c]	17.5	17.7	18.4	16.4	17.6	18.5
Days 22 to 24[d]	18.0	18.8	18.7	17.7	18.2	20.3
Days 0 to 24[d]	16.1	16.3	16.5	15.2	16.1	17.6
Gain/feed[b]	.317	.340	.318	.315	.333	.316

[a] Quadratic effect of arginine at .64% lysine (1, 3 vs 2), P<.10.
[b] Quadratic effect of arginine at .64% lysine (1, 3 vs 2), P<.05.
[c] Linear effect of arginine at .92% lysine (4 vs 6), P<.05.
[d] Linear effect of arginine at .92% lysine (4 vs 6), P<.01.

Arginine had a significant quadratic effect on the gain/feed of rats fed .64% lysine and tended to have a similar effect ($P<.11$) in rats fed .92% lysine. Although weekly gain/feed values are not presented in table 7, calculations by the author indicate that the gain/feed pattern was consistent throughout the trial.

Lysine main effects for daily gain, feed intake and gain/feed of rats in Exp. A are presented in table 8. There were no significant lysine main effects on any of the performance variables measured. However, supplemental lysine appeared to depress the daily gain and feed intake of rats fed .41% arginine (table 7). Supplemental arginine corrected the effects of lysine on growth and feed intake, and rats fed the high lysine, high arginine diet had the highest feed intake of any group.

The effects of arginine on rat plasma amino acid levels are presented in table 9. Supplemental arginine caused a linear ($P<.01$) and quadratic ($P<.05$) increase in plasma arginine levels of rats fed .64% or .92% lysine. Increasing dietary arginine caused an increase ($P<.01$) in plasma ornithine levels of rats fed .64% lysine but had no effect on plasma ornithine levels of rats fed .92% lysine.

Increased dietary arginine reduced ($P<.05$) plasma leucine levels and tended to reduce ($P<.10$) plasma lysine and valine levels of rats fed .66% lysine. In rats fed .92% lysine, excess arginine reduced ($P<.01$) the plasma levels of lysine, cystine, methionine, isoleucine and leucine and tended to reduce ($P<.10$) plasma levels of valine and phenylalanine. Plasma tryptophan and threonine levels were unaffected by dietary arginine.

TABLE 8. LYSINE MAIN EFFECTS FOR DAILY GAIN, FEED INTAKE AND FEED EFFICIENCY OF RATS IN EXPERIMENT A[a,b]

Variable	Lysine level .64	Lysine level .92
Average daily gain, g		
Days 0 to 7	6.0	5.9
Days 8 to 14	5.5	5.2
Days 15 to 24	4.7	4.8
Days 0 to 24	5.3	5.2
Average daily feed intake, g		
Day 1	12.8	12.8
Day 2	11.7	12.2
Day 3	12.4	12.5
Days 4 to 5	14.5	14.2
Days 6 to 7	14.6	15.0
Days 8 to 14	16.5	16.7
Days 15 to 21	17.9	17.5
Days 22 to 24	18.5	18.7
Days 0 to 24	16.3	16.3
Gain/feed	.325	.321

[a] Rats were fed the test diets ad libitum for 24 d.
[b] There were no significant lysine main effects (P>.10).

TABLE 9. EFFECT OF ARGININE ON PLASMA AMINO ACID LEVELS OF RATS FED AD LIBITUM FOR 24 DAYS (EXPERIMENT A)

Lysine, %	.64			.92		
Arginine, %	.41	1.12	2.39	.41	1.14	2.39
Arginine/lysine	.64	1.75	3.73	.45	1.24	2.60
Treatment no.	1	2	3	4	5	6
	Plasma amino acid levels, mg/100 ml plasma					
Arginine[cegh]	1.83	1.84	3.61	1.87	2.25	4.39
Histidine	--	--	--	--	--	--
Lysine[ag]	7.34	6.58	6.38	8.79	7.53	6.81
Ornithine[c]	.68	.75	1.33	.76	.71	.85
Citrulline	.40	.29	.45	.33	.29	.27
Cystine[gi]	1.57	1.50	1.40	3.82	1.35	1.43
Methionine[g]	.73	.64	.62	.83	.64	.51
Isoleucine[g]	1.00	1.05	.97	1.06	.89	.74
Leucine[bg]	1.61	1.59	1.26	1.61	1.35	1.13
Valine[af]	2.49	2.36	2.00	2.16	2.09	1.69
Phenylalanine[df]	1.15	1.27	1.06	1.14	1.10	.92
Threonine	3.95	3.60	3.41	3.78	3.45	3.59
Tryptophan	3.02	2.60	2.37	3.16	2.58	2.60

[a] Arginine linear effect at .64% lysine (1 vs 3), $P<.10$.
[b] Arginine linear effect at .64% lysine (1 vs 3), $P<.05$.
[c] Arginine linear effect at .64% lysine (1 vs 3), $P<.01$.
[d] Arginine quadratic effect at .64% lysine (1, 3 vs 2), $P<.10$.
[e] Arginine quadratic effect at .64% lysine (1, 3 vs 2), $P<.05$.
[f] Arginine linear effect at .92% lysine (4 vs 6), $P<.10$.
[g] Arginine linear effect at .92% lysine (4 vs 6), $P<.01$.
[h] Arginine quadratic effect at .92% lysine (4, 6 vs 5), $P<.05$.
[i] Arginine quadratic effect at .92% lysine (4, 6 vs 5), $P<.01$.

Lysine main effects on rat plasma amino acid levels in Exp. A are presented in table 10. Plasma lysine levels were increased ($P<.01$) by lysine supplementation to the diet. Lysine supplementation also increased plasma levels of cystine ($P<.01$) and tended to increase plasma arginine levels ($P<.10$). The remaining plasma amino acids were not affected by lysine supplementation to the diet.

Experiment B

Experiment B was designed to detect competition between lysine and arginine for absorption from the small intestine. Blood samples were taken from the rats 4 h after the end of the 2-h meal period. The hypothesis was that if arginine did reduce lysine absorption it would be reflected by a decrease in plasma lysine levels.

Results from Exp. B are presented in table 11. Feed intake was not equalized between treatment groups, and rats fed the 2.39% arginine diet tended to consume less feed during the 2-h meal period ($P<.10$).

Plasma lysine was not affected by excess dietary arginine, indicating that arginine was not interfering with lysine absorption to an extent measurable in this experiment.

Plasma arginine and ornithine levels were increased linearly by increased dietary arginine ($P<.001$), and plasma histidine levels tended to be increased ($P<.10$). The other plasma amino acids were not affected by treatment.

TABLE 10. LYSINE MAIN EFFECTS FOR RAT PLASMA AMINO ACID LEVELS IN EXPERIMENT A[a]

Variable	Lysine level .64	Lysine level .92
Plasma amino acid levels, mg/100 ml plasma		
Arginine[b]	2.42	2.84
Histidine	--	--
Lysine[d]	6.77	7.71
Ornithine	.92	.77
Citrulline	.38	.30
Cystine[d]	1.49	2.20
Methionine	.66	.66
Isoleucine	1.00	.90
Leucine	1.49	1.37
Valine[c]	2.28	1.98
Phenylalanine[b]	1.16	1.05
Threonine	3.65	3.61
Tryptophan	2.66	2.78

[a] Rats were fed ad libitum for 24 d.
[b] Lysine effect ($P<.10$).
[c] Lysine effect ($P<.05$).
[d] Lysine effect ($P<.01$).

TABLE 11. EFFECT OF ARGININE ON FEED INTAKE AND PLASMA AMINO ACID LEVELS OF RATS FED TEST DIETS FOR 2 HOURS (EXPERIMENT B)[a]

Lysine, %	.64		
Arginine, %	.41	1 12	2.39
Arginine/lysine	.64	1.75	3.37
Adjustment period gain, g	22.0	22.9	22.0
Adjustment period feed intake, g	72.0	71.2	71.1
Test diet feed intake, g[b]	5.8	5.6	4.6
Plasma amino acid levels, mg/100 ml plasma			
Arginine[c]	2.67	4.91	7.87
Histidine[b]	1.37	1.74	1.72
Lysine	7.70	7.87	8.32
Ornithine[c]	.97	1.28	1.85
Citrulline	--	--	--
Cystine	--	--	--
Methionine	.96	1.00	1.13
Isoleucine	1.64	1.59	1.49
Leucine	1.87	1.98	2.34
Valine	2.28	2.31	2.83
Phenylalanine	1.44	1.49	1.73
Threonine	5.18	5.37	6.23
Tryptophan	2.28	2.40	2.32

[a] Blood samples were taken 4 h after the end of the 2-h meal period.
[b] Linear effect of arginine ($P<.10$).
[c] Linear effect of arginine ($P<.001$).

Experiment C

A second experiment was conducted to evaluate the effects of arginine on postprandial plasma amino acid levels. In Exp. C, rats were fed their respective test diets in four daily meals after having been meal-fed a stock diet for 20 d. Body weight changes and feed intakes of the rats are listed in table 12.

All rats lost weight during the experimental period, but these losses varied widely and there were no significant treatment differences.

Feed consumption was not measured during the 21-d adaptation period, so it was not possible to determine the effectiveness of the adaptation period in increasing feed intake during meal feeding. Rats in Exp. B consumed 4.07% of body weight in feed, while rats in Exp. C consumed an average of 2.79% of body weight in feed during the meal periods. Differences in body weight between Exp. B and C (131.0 vs 292.4 g, respectively) made direct comparison of relative feed intakes difficult.

In Exp. C, increasing arginine caused a linear decrease ($P<.03$) in feed consumption of rats fed .66% and .86% lysine (table 12). This agreed with the results from Exp. B, where arginine also had a negative effect on feed consumption. Dietary lysine level did not affect feed consumption in Exp. C (table 13).

The effects of arginine on rat plasma amino acid levels in Exp. C are presented in table 14. Increasing dietary arginine resulted in a significant increase in plasma arginine, ornithine, citrulline, lysine and phenylalanine levels of rats fed .66% lysine. Plasma

TABLE 12. EFFECT OF ARGININE ON BODY WEIGHT CHANGE AND FEED INTAKE OF RATS MEAL-FED TEST DIETS FOR FOUR DAYS (EXPERIMENT C)[a]

Lysine, %	.66			.86		
Arginine, %	.38	2.57	5.09	.31	3.18	5.16
Arginine/lysine	.56	3.89	7.71	.36	3.70	6.00
Treatment no.	1	2	3	4	5	6
Body wt loss, g	19.5	25.3	25.7	22.8	27.5	27.8
Average daily feed intake, g						
Day 1[c]	7.5	7.8	6.5	10.1	7.0	4.4
Day 2[c]	9.1	8.6	7.5	10.8	7.7	7.6
Day 3	10.1	8.5	8.4	9.0	8.5	7.5
Day 4[b]	10.5	7.7	7.4	9.2	7.6	8.5
Days 1 to 4[bc]	9.3	7.7	7.4	9.8	7.7	7.0

[a] Two-hour meal periods; blood samples were taken 4 h after the end of the last meal period.
[b] Linear effect of arginine at .66% lysine (1 vs 3), $P<.03$.
[c] Linear effect of arginine at .86% lysine (4 vs 6), $P<.02$.

TABLE 13. LYSINE MAIN EFFECTS FOR EXPERIMENT C[a]

Variable	Lysine level .66	Lysine level .86
Body wt loss, g	23.5	26.1
Average daily feed intake, g		
Day 1	7.2	7.2
Day 2	8.4	8.7
Day 3	9.0	8.3
Day 4	8.5	8.4
Days 1 to 4	8.1	8.1
Plasma amino acid levels, mg/100 ml plasma		
Arginine	5.33	5.50
Histidine	--	--
Lysine[c]	4.89	6.96
Ornithine	2.00	2.24
Citrulline	.28	.28
Cystine	--	--
Methionine	.67	.73
Isoleucine[b]	.76	.96
Leucine	1.26	1.45
Valine	1.73	1.97
Phenylalanine	1.17	1.17
Threonine	3.23	3.34
Tryptophan	3.52	3.62

[a] Test diets fed 2 h daily for 4 d; blood samples taken 4 h after the end of the last meal period.
[b] Lysine effect ($P<.05$).
[c] Lysine effect ($P<.01$).

TABLE 14. EFFECT OF ARGININE ON PLASMA AMINO ACID LEVELS OF RATS MEAL-FED TEST DIETS FOR FOUR DAYS (EXPERIMENT C)[a]

Lysine, %	.66			.86		
Arginine, %	.38	2.57	5.09	.31	3.18	5.16
Arginine/lysine	.56	3.89	7.71	.36	3.70	6.00
Treatment no.	1	2	3	4	5	6
	Plasma amino acid levels, mg/100 ml plasma					
Arginine[dfg]	1.48	5.54	8.98	1.74	4.71	10.04
Histidine	--	--	--	--	--	--
Lysine[c]	3.95	5.01	5.71	7.00	7.16	6.70
Ornithine[dfh]	.51	2.08	3.42	.99	1.69	4.03
Citrulline[ce]	.17	.28	.38	.21	.31	.34
Cystine	--	--	--	--	--	--
Methionine	.57	.70	.76	.82	.69	.69
Isoleucine[beg]	.61	.76	.91	.92	.83	1.13
Leucine[b]	.94	1.24	1.60	1.52	1.44	1.41
Valine	1.45	1.77	1.98	1.99	2.00	1.93
Phenylalanine[ch]	1.03	1.14	1.34	1.24	1.00	1.27
Threonine	3.14	3.12	3.43	3.43	3.16	3.42
Tryptophan[h]	3.32	3.51	3.73	3.60	2.97	4.28

[a] Two-hour meal periods; blood samples were taken 4 h after the end of the last meal period.
[b] Arginine linear effect at .66% lysine (1 vs 3), $P<.10$.
[c] Arginine linear effect at .66% lysine (1 vs 3), $P<.05$.
[d] Arginine linear effect at .66% lysine (1 vs 3), $P<.01$.
[e] Arginine linear effect at .86% lysine (4 vs 6), $P<.10$.
[f] Arginine linear effect at .86% lysine (4 vs 6), $P<.01$.
[g] Arginine quadratic effect at .86% lysine (4, 6 vs 5), $P<.10$.
[h] Arginine quadratic effect at .86% lysine (4, 6 vs 5), $P<.05$.

arginine and ornithine were increased (P<.01) and plasma isoleucine and citrulline tended to be increased (P<.10) by excess arginine in rats fed .86% lysine. Dietary arginine had a quadratic effect (P<.05) on plasma phenylalanine and tryptophan levels of rats fed .86% lysine.

The lysine main effects on plasma amino acid levels in Exp. C are presented in table 13. Plasma lysine and isoleucine were increased by lysine supplementation to the diet. There were no other significant lysine main effects on rat plasma amino acid levels in Exp. C.

Experiments With Swine

Two experiments were conducted with swine to determine the effects of excess arginine on pig performance and plasma amino acid levels. The basal diet for Exp. 1 contained corn, oats groats, dried skim milk and fish meal. The basal diet for Exp. 2 was similar except that the oats groats were removed and replaced with corn and dried skim milk. Diets for Exp. 1 contained 10.0% dried skim milk, while Exp. 2 diets contained 19.56% dried skim milk.

Experiment 1. Arginine effects for Exp. 1 are presented in table 15. Daily gain data of two pigs from treatment 5 and one pig from treatment 6 were removed from the study. The three pigs had daily gains that were less than half that of any other pig in the study. It was felt that their poor performance was not due to treatment and that removal of their gain data would allow a more accurate assessment of treatment effects. Daily feed intake and gain/feed data were measured on a pen basis. Since adjustment of these data would require arbitrary

TABLE 15. EFFECT OF ARGININE ON PERFORMANCE AND PLASMA AMINO ACID LEVELS OF PIGS FED AD LIBITUM FOR 28 DAYS (EXPERIMENT 1)

Lysine, %	1.03			1.26		
Arginine, %	.94	1.34	1.63	.94	1.24	1.62
Arginine/lysine	.19	1.30	1.58	.75	.98	1.29
Treatment no.	1	2	3	4	5	6
	Performance					
Daily gain, g	323	316	297	362	338	373
Daily feed intake, g[e]	648	637	630	683	591	684
Gain/feed[a]	.499	.496	.469	.528	.515	.524
	Plasma amino acid levels, mg/100 ml plasma					
Arginine[ac]	3.47	4.10	4.75	3.02	3.92	5.32
Histidine	3.23	3.07	2.64	2.42	2.44	3.00
Lysine[d]	1.96	1.94	1.76	4.92	4.59	4.20
Ornithine[ac]	2.69	2.99	3.92	2.46	2.95	3.83
Citrulline	1.80	1.62	1.74	1.47	1.45	1.48
Cystine	--	--	--	--	--	--
Methionine[a]	1.00	.88	.82	.79	.73	.75
Isoleucine	2.01	1.92	1.96	1.76	1.69	1.82
Leucine	3.39	3.23	3.18	3.23	3.04	3.29
Valine	4.54	4.37	4.41	4.01	3.88	4.12
Phenylalanine	3.03	2.99	2.92	2.55	2.44	2.64
Threonine[ab]	4.68	3.43	3.77	1.40	1.38	1.38
Tryptophan	--	--	--	--	--	--

[a] Arginine linear effect at 1.03% lysine (1 vs 3), P<.05.
[b] Arginine quadratic effect at 1.03% lysine (1, 3 vs 2), P<.05.
[c] Arginine linear effect at 1.26% lysine (4 vs 6), P<.01.
[d] Arginine linear effect at 1.26% lysine (4 vs 6), P<.06.
[e] Arginine quadratic effect at 1.26% lysine (4, 6 vs 5), P<.05.

estimation of feed intake, the data were not adjusted and do contain data from the three poor-performing pigs.

There were not significant effects of arginine on average daily gain. Dietary arginine had a significant quadratic effect on feed intake in pigs fed 1.26% lysine. This was probably due to the low feed intake of the two poor-performing pigs on treatment 5 mentioned previously. Overall, arginine did not affect feed intake in Exp. 1.

Pigs fed 1.03% lysine were less efficient ($P<.05$) when dietary arginine was increased to 1.63%. This effect was not apparent in pigs fed 1.26% lysine, indicating that supplemental lysine did correct the adverse effect of arginine on feed efficiency.

At both levels of dietary lysine, increasing levels of dietary arginine caused a linear increase in plasma arginine and ornithine levels. Plasma lysine was decreased ($P<.06$) by dietary arginine in pigs fed 1.26% lysine but not in pigs fed 1.03% lysine.

Increased dietary arginine caused a linear ($P<.05$) and quadratic ($P<.05$) decrease in plasma threonine levels and a linear decrease ($P<.05$) in plasma methionine levels of pigs fed 1.03% lysine. Arginine did not affect plasma threonine and methionine levels in pigs fed 1.26% lysine. The remaining plasma amino acids were not affected by dietary arginine content.

The lysine main effects for Exp. 1 are presented in table 16. Average daily gain was improved ($P<.05$) and gain/feed tended to be improved ($P<.12$) by increased dietary lysine. Lysine supplementation had no effect on feed intake.

TABLE 16. LYSINE MAIN EFFECTS FOR EXPERIMENT 1[a]

Variable	Lysine level 1.03	1.26
Average daily gain, g[b]	312	358
Average daily feed intake, g	638	652
Gain/feed[d]	.488	.522
Plasma amino acid levels, mg/100 ml plasma		
Arginine	4.11	4.08
Histidine[c]	2.98	2.62
Lysine[b]	1.89	4.57
Ornithine	3.20	3.08
Citrulline[b]	1.72	1.47
Cystine	--	--
Methionine[b]	.90	.76
Isoleucine[c]	1.96	1.75
Leucine	3.27	3.19
Valine[b]	4.44	4.00
Phenylalanine[b]	2.98	2.55
Threonine[b]	3.96	1.38
Tryptophan	--	--

[a] Twenty-eight day growth trial.
[b] Lysine effect ($P<.05$).
[c] Lysine effect ($P<.07$).
[d] Lysine effect ($P<.12$).

Plasma levels of histidine, citrulline, methionine, isoleucine, valine, phenylalanine and threonine were decreased when the diet was supplemented with lysine. Plasma lysine was increased in pigs fed 1.26% lysine. Plasma arginine, ornithine and leucine were unaffected by lysine supplementation.

Experiment 2. The results of Exp. 2 are found in table 17. The daily gain data of two pigs from treatment 4, one pig from treatment 3 and one pig from treatment 2 were removed from the study because of extremely poor performance. The justification for data removal was discussed in the results of Exp. 1.

Average daily gain was unaffected by arginine and lysine supplementation. Pigs fed .92% lysine and 1.10% arginine tended to have increased feed intake as compared to pigs fed .92% lysine and either .72% or 1.61% arginine, but the quadratic effect was not statistically significant. Lysine supplementation reduced ($P<.01$) the feed intake of pigs in Exp. 2.

The slightly increased feed intake of pigs fed 1.10% arginine resulted in a significant quadratic effect of arginine on gain/feed. Pigs fed 1.10% arginine were less efficient ($P<.05$) than those fed .72% or 1.61% arginine. Pigs fed the 1.10% lysine control diet were more efficient ($P<.01$) than pigs fed diets containing .92% lysine.

Increased dietary arginine caused a linear increase ($P<.05$) in plasma arginine and a linear and quadratic increase ($P<.05$) in plasma ornithine. Plasma lysine was not affected by arginine supplementation to the diet. Arginine decreased plasma levels of threonine and

TABLE 17. EFFECT OF ARGININE ON PERFORMANCE AND PLASMA AMINO ACID LEVELS OF PIGS FED AD LIBITUM FOR 26 DAYS (EXPERIMENT 2)[a]

Lysine, %	.92			1.10
Arginine, %	.72	1.10	1.61	.70
Arginine/lysine	.78	1.20	1.75	.64
Treatment no.	1	2	3	4
Performance				
Average daily gain, g	461	453	474	457
Average daily feed intake, g[d]	833	865	832	737
Gain/feed[be]	.552	.508	.548	.573
Plasma amino acid levels, mg/100 ml plasma				
Arginine[ad]	2.60	5.72	7.32	1.87
Histidine[d]	2.32	2.26	2.07	1.57
Lysine[d]	2.69	2.61	2.62	4.50
Ornithine[abd]	1.73	3.26	3.99	1.54
Citrulline[d]	1.53	1.58	1.51	1.33
Cystine[d]	3.11	3.09	3.06	2.82
Methionine[ad]	1.45	1.30	1.16	1.05
Isoleucine[c]	2.71	2.53	2.51	2.23
Leucine	5.10	5.02	4.65	4.77
Valine[d]	4.55	4.77	4.34	3.09
Phenylalanine[bd]	2.69	2.89	2.55	1.71
Threonine[ac]	9.74	8.12	6.21	5.84
Tryptophan	5.85	5.75	5.94	5.44

[a] Arginine linear effect (1 vs 3), $P<.05$.
[b] Arginine quadratic effect (1, 3 vs 2), $P<.05$.
[c] Lysine effect (1, 2, 3 vs 4), $P<.08$.
[d] Lysine effect (1, 2, 3 vs 4), $P<.05$.
[e] Lysine effect (1, 2, 3 vs 4), $P<.01$.

methionine ($P<.05$), which was in agreement with the results from Exp. 1. Plasma levels of the other amino acids were not affected by arginine supplementation to the diet.

Plasma histidine, citrulline, cystine, methionine, valine and phenylalanine were decreased ($P<.05$) and plasma isoleucine and threonine tended to be decreased ($P<.08$) in pigs fed the high lysine control diet. Plasma lysine was increased by lysine supplementation to the diet.

The lysine effect was also significant for plasma arginine and ornithine, but these results were confounded with the effect of arginine and no conclusion can be drawn concerning the effects of lysine supplementation on plasma arginine and ornithine.

DISCUSSION

Experiment A

Feed intake of the rats was measured daily for the first few days of Exp. A in order to determine rapid changes in feed intake. The results indicate that excess arginine had no adverse effect on either daily gain or feed intake. In fact, there was a positive response to arginine supplementation in rats fed .92% lysine. Arginine had an inconsistent effect on gain/feed; rats fed .41% or 2.39% arginine had similar gain/feed, while rats fed 1.13% arginine (average) had a slightly higher gain/feed.

Sauberlich (1961) found that a 5% arginine addition to a 6% casein diet depressed rat growth by 44%. Harper et al. (1966) noted a growth depression when 4% arginine was added to a 6% casein diet, and this adverse effect was corrected when the diets were supplemented with the limiting amino acids (methionine and tryptophan). Apparently the growth depression was due to an imbalance created by excess arginine and not to a specific effect of arginine on lysine utilization.

Experiment A indicated that rats fed a 9% casein diet supplemented with cystine, threonine and isoleucine can tolerate up to 1.61% arginine without adverse effect. The lack of a negative effect of arginine may be due to the lower levels of arginine used and to the slightly higher protein content of the basal diet used in this experiment as compared with other studies.

Lysine supplementation had a negative effect on growth and feed intake of rats fed .41% arginine (table 7). Thus, lysine was not

growth limiting in the diet and lysine supplementation appeared to result in a mild lysine imbalance.

Arginine supplementation completely corrected the adverse effects of lysine, indicating that arginine was the growth limiting amino acid or that lysine had a specific effect on arginine utilization. The lack of an effect of lysine on plasma arginine levels (tables 9 and 10) is evidence against a specific lysine effect on arginine utilization.

The most likely explanation for the ability of arginine to correct the lysine-induced growth depression is simply that arginine was the limiting amino acid in the basal diet and that lysine supplementation created a mild imbalance. Amino acid imbalances can be corrected by supplementing the limiting amino acid to the diet (Harper et al., 1970). The 9% casein diet contained .41% arginine, which is the amount required by the rat as determined by Stockland and Meade (1970). Jones et al. (1966) indicated that arginine may be the limiting amino acid in casein for growing rats.

In a study by Hevia et al. (1980), rats fed a 15% casein diet exhibited fatty livers, depressed growth and reduced feed intake when 5% lysine was added to the diet. These symptoms were alleviated by adding 1% arginine to the diet. Jones et al. (1966) observed a 6.0% depression in rat growth when 1.5% lysine was added to an 18% casein diet. Supplemental arginine corrected this growth depression. In the present experiment, a .28% lysine supplement caused a 5.9% growth depression which was corrected by adding arginine. The lower protein content of the diets in Exp. A (9% casein) as compared to the protein

levels used by Jones et al. (1966) and Hevia et al. (1980) probably contributed to the rats' increased sensitivity to the lysine supplement, since rats are more susceptible to amino acid imbalance when fed low protein diets (Harper et al., 1970).

Other researchers have found rats to be less susceptible to a lysine imbalance. Stockland et al. (1970a) used a crystalline amino acid diet (10% protein equivalent) to investigate the lysine requirement of 85-g rats. Rat growth was maximized by .64% lysine and there were no adverse effects when lysine was fed at levels up to 1.44%. Peng (1979) fed 5% casein diets and observed maximal rat growth with .58% lysine, while up to 1.10% lysine produced no depression in rat growth.

The decrease in plasma lysine resulting from arginine supplementation suggests a specific effect of arginine on lysine utilization. However, arginine supplementation also reduced plasma leucine and valine levels in rats fed .64% lysine and reduced plasma cystine, methionine, isoleucine, leucine, valine and phenylalanine levels in rats fed .92% lysine. Arginine appeared to have a general effect on plasma amino acid levels rather than a specific effect on plasma lysine.

The decline in plasma amino acid levels due to arginine supplementation is difficult to rationalize from the performance data. Arginine supplementation increased growth in rats fed .92% lysine, which would cause increased utilization of plasma amino acids. However, the increased growth was accompanied by increased feed intake and there was no consistent effect of arginine on feed efficiency. Thus, the decline

in plasma amino acids cannot be related to changes in growth, feed intake or efficiency of feed utilization.

One possible explanation is that arginine caused an increase in the breakdown of amino acids by stimulating the activity of enzymes involved in amino acid catabolism. This statement is offered as hypothesis only and further detailed investigation would be required to fully explain the effects of arginine on plasma amino acid levels.

Lysine supplementation had little effect on plasma amino acid levels other than to increase plasma lysine. The increase in plasma cystine due to lysine supplementation resulted from the very high levels of cystine in rats fed treatment 4 (table 9). The reason for these high levels is unknown. Lysine caused a slight decrease in plasma valine and phenylalanine. These changes were small and their importance is questionable.

Experiments B and C

Increased dietary arginine resulted in decreased feed intake by rats in Exp. B and C. Arginine may have exerted an effect on appetite via the plasma amino acid pattern (amino acid imbalance). This explanation is unlikely since rats that were fed similar diets ad libitum in Exp. A did not decrease their feed intake. Also, arginine caused no consistent depression in the plasma level of any amino acid in Exp. B or C.

A more likely explanation of the decreased feed intake is that adding arginine hydrochloride to the diet increased the osmotic pressure

of the stomach contents, drawing water into the stomach and causing satiation by stomach distension. This effect is much more critical in meal-fed animals as compared to ad libitum-fed animals, since stomach distension is likely the limiting factor of feed intake in animals fed only once daily.

Increasing the arginine content of the diet from .32% (average) to 5.13% (average) decreased feed consumption by 1.9 g in rats fed .66% lysine and by 2.8 g in rats fed .86% lysine (table 12). Although the experimental design did not allow a test for interaction, it appeared that lysine supplementation aggravated the feed intake depression caused by arginine supplementation. This observation is consistent with the theory that an increased number of free amino acids in the stomach may reduce feed intake by causing fluid accumulation and stomach distension.

Chen et al. (1962) observed an increase in the stomach moisture content of rats fed a crystalline amino acid diet versus rats fed a casein diet. Itoh et al. (1974) found stomach distension to be the main factor causing reduced feed intake in rats fed crystalline amino acid diets.

In Exp. B and C, increased dietary arginine caused a marked increase in plasma arginine and ornithine, and in Exp. C lysine supplementation caused an increase in plasma lysine. These changes indicated that the plasma amino acid levels were sensitive to changes in the level of available amino acids in the diet. Thus, plasma lysine levels should have reflected any change in lysine absorption caused by excess arginine. Since plasma lysine was unaffected by dietary arginine content in

Exp. B and C, the conclusion was that arginine did not affect lysine absorption in these experiments.

Experiment C was the only case in this series of rat and swine experiments where increased dietary arginine caused an increase in plasma citrulline. Increased ornithine and an increase in carbamoyl phosphate synthesis from ammonia are necessary for an increase in citrulline synthesis to occur (Lehninger, 1970). In rat Exp. A and swine Exp. 1 and 2, arginine additions to the diet caused an increase in plasma ornithine levels, but apparently there was no excess carbamoyl phosphate available and thus no increase in citrulline synthesis occurred. It is important to note that in those experiments the animals were on a positive plane of nutrition and there would be minimal amino acid catabolism. Carbamoyl phosphate production from amino acid breakdown would be low.

In Exp. C, the rats were meal-fed and were unable to maintain body weight on this dietary regimen. The rats lost an average of 24.8 g of body weight during the experimental period, indicating that considerable protein catabolism was occurring. This would result in a higher level of carbamoyl phosphate synthesis. When plasma ornithine levels were increased by feeding excess arginine, the extra carbamoyl phosphate was present to allow increased citrulline synthesis.

Increased dietary arginine had statistically significant effects on plasma phenylalanine and tryptophan in Exp. C. Lysine supplementation increased plasma isoleucine levels in Exp. C. These changes were small

and(or) quadratic in nature and are difficult to rationalize on the basis of treatment effects.

Experiments 1 and 2

The lysine main effects for Exp. 1 presented in table 16 indicate that lysine was the limiting amino acid in the diets containing 1.03% lysine. The reduced plasma amino acid levels of pigs fed the lysine-supplemented diets indicate increased utilization of these amino acids for growth and protein synthesis, since pigs fed 1.26% lysine had higher daily gains and tended to have a higher gain/feed than pigs fed 1.03% lysine. Bravo et al. (1970) and Stockland et al. (1970b) also noted a general reduction in plasma essential amino acid levels when the limiting amino acid was supplemented to the diet.

The reduced plasma citrulline levels are an indication that lysine supplementation reduced the amount of excess plasma amino acids available for deamination, which reduced the number of amino groups available for carbamoyl phosphate synthesis. Bravo et al. (1970) and Taylor et al. (1981) observed a decrease in blood urea levels of swine when the limiting amino acid was supplemented to the diet.

In Exp. 1, arginine caused a linear decrease ($P<.06$) in the plasma lysine levels of pigs fed 1.26% lysine, but there was no reduction in pig performance. This response indicates that in pigs fed 1.26% lysine, arginine may have exerted a specific effect on plasma lysine levels but there was enough excess lysine available to the pig to prevent any decrease in performance.

These results agree with the findings of Easter and Baker (1977). Their data indicate that lysine must be limiting in the diet before arginine will have a detrimental effect on pig performance. In the present study, the performance of pigs fed excess lysine was not affected by increased dietary arginine, while pigs fed diets limiting in lysine had reduced gain/feed when fed excess arginine.

In pigs fed 1.03% lysine, plasma lysine levels were low and were not affected by dietary arginine content. Data from Mitchell et al. (1968) indicated that when an amino acid becomes limiting in the diet, a further reduction in the dietary level of that amino acid will not affect its plasma level. Southern and Baker (1982) observed that arginine caused a much larger decrease in the plasma lysine levels of pigs fed 1.58% lysine versus pigs fed 1.08% lysine. There was no response in pig performance to lysine supplementation in that study, indicating that 1.08% lysine was in excess of the pigs' requirement.

Thus the lack of a reduction in plasma lysine levels due to arginine supplementation in pigs fed 1.03% lysine is not surprising, since plasma lysine was already at baseline levels. Although plasma lysine levels were not affected, arginine still had an effect on lysine utilization in pigs fed 1.03% lysine, since those pigs had a reduced gain/feed when fed 1.63% arginine. The mechanism for the effect of arginine on lysine utilization is unknown. Southern and Baker (1982) found that only 2% of the total lysine intake was excreted in the urine when pigs were fed 2.8% arginine. Thus, it is doubtful that urinary lysine excretion played a role in the arginine-lysine antagonism observed in Exp. 1.

Results from Exp. 2 indicate that lysine was the growth limiting amino acid in pigs fed .92% lysine. Lysine supplementation to the diet resulted in an improvement in gain/feed and a general reduction in plasma essential amino acid levels. Plasma citrulline levels were also reduced by lysine supplementation. These results agree with the response to lysine supplementation observed in Exp. 1. Plasma leucine levels were not affected by lysine supplementation in Exp. 1 or 2. Evidently leucine is not utilized to any great extent in the growth response to lysine supplementation.

Increased dietary arginine had no effect on pig performance or plasma lysine levels in Exp. 2. This was in contrast to the results from Exp. 1, despite the fact that the diets in Exp. 2 contained less lysine and had arginine to lysine ratios of up to 1.75:1, whereas diets in Exp. 1 had arginine to lysine ratios of up to 1.58:1.

The lack of an effect of arginine on pig performance in Exp. 2 as compared to Exp. 1 may be due to the difference in lysine intake and degree of lysine deficiency between the two trials. Due to differences in feed intake, pigs fed .92% lysine in Exp. 2 consumed 17.7% more total lysine than pigs fed 1.03% lysine in Exp. 1. Plasma lysine levels of pigs fed .92% lysine in Exp. 2 appeared to be higher than the plasma lysine levels of pigs fed 1.03% lysine in Exp. 1. The improved lysine status of pigs in Exp. 2 could have minimized the growth-depressing effects of excess arginine.

This explanation is not entirely satisfactory since lysine supplementation in Exp. 2 did result in an improved gain/feed,

indicating that the pigs fed .92% lysine were at least mildly deficient in lysine. The conclusion was that excess arginine had no effect on lysine utilization or pig performance in Exp. 2, even though a lysine-deficient basal diet was used.

A consistent observation from Exp. 1 and 2 was that increasing levels of dietary arginine decreased the plasma threonine and methionine levels of pigs in Exp. 2 and of pigs fed 1.03% lysine in Exp. 1. This effect was not apparent in pigs fed 1.26% lysine in Exp. 1, because the plasma threonine and methionine levels were already at or near baseline levels. The lysine supplementation reduced plasma threonine and methionine levels to the point where it would be difficult to decrease them further.

The effect of arginine on plasma threonine and methionine levels in Exp. 1 and 2 appeared to be a specific effect on those amino acids since arginine did not reduce the plasma levels of the other amino acids. In rat Exp. A, excess arginine reduced the plasma methionine levels of rats fed .92% lysine, but this result appeared to be part of an overall effect of arginine on rat plasma amino acid levels. Zimmerman and Scott (1965) noted a decline in the plasma threonine, glycine and lysine levels of chicks fed superoptimal levels of dietary arginine, but plasma methionine was not affected. Further research of this relationship in swine may be merited from the standpoint of establishing basic knowledge, but its importance in practical swine nutrition is probably minimal.

SUMMARY

Three experiments with rats and two experiments with weaned pigs were conducted to determine the effects of excess arginine on animal performance and plasma amino acid levels. Of special interest was the effect of arginine on lysine utilization.

Treatments for the 24-d rat growth trial consisted of two lysine levels (.64% and .92%) and three arginine levels (.41%, 1.13% and 2.39%) in a 2 x 3 factorial arrangement. Excess arginine had no adverse effect on rat growth, and in rats fed .92% lysine there was a positive response to arginine supplementation. Lysine appeared to cause a mild imbalance which was corrected by increasing dietary arginine. Excess arginine caused a general reduction in plasma amino acid levels of rats fed .92% lysine. These reductions could not be related to changes in growth, feed intake or feed efficiency.

Two short-term feeding trials were conducted to determine if arginine was interfering with lysine absorption from the small intestine. Rats were fed the test diets in daily 2-h meal periods for either 1 or 4 d, and blood samples were taken 4 h after the end of the last meal period. Plasma arginine and ornithine levels were readily affected by dietary arginine content. Plasma lysine levels were unaffected by dietary levels of up to 5.16%, indicating the excess arginine had no effect on lysine absorption from the small intestine of rats.

In the first 28-d swine growth trial, two lysine levels (1.03% and 1.26%) and three arginine levels (.94%, 1.29% and 1.63%) were used in a 2 x 3 factorial arrangement. Lysine supplementation improved daily

gains, tended to improve feed efficiency and caused a general reduction in plasma essential amino acid levels, indicating that lysine was the limiting amino acid in the basal diet. Arginine had no effect on daily gain or feed intake, but pigs fed 1.03% lysine and 1.63% arginine had reduced gain/feed. Arginine did not affect gain/feed in pigs fed 1.26% lysine, indicating that lysine supplementation corrected the adverse effect of arginine on feed efficiency.

Plasma lysine levels of pigs fed 1.03% dietary lysine were at baseline levels and were not affected by arginine supplementation. In pigs fed 1.26% lysine, excess arginine caused a reduction in plasma lysine levels. Overall, the results from the first trial indicated that arginine did affect lysine utilization and that 1.63% arginine reduced the performance of weaned pigs fed lysine-deficient diets.

Treatments for the second 26-d swine growth trial consisted of three diets containing .92% lysine and either .72%, 1.10% or 1.61% arginine. The fourth diet was a positive lysine control (1.10% lysine, .70% arginine). Lysine was the limiting amino acid in the basal diet, but arginine had no effect on daily gain, daily feed intake, gain/feed or plasma lysine levels.

Conclusions from the swine research were that large excesses of arginine may affect lysine utilization, but pig performance was affected only when excess arginine was combined with a lysine deficiency. The arginine levels found in normal swine diets had no effect on lysine utilization or pig performance in these experiments. Thus, there was no justification for reformulating diets to lower the arginine to lysine ratio.

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APPENDIX

NOTE: The mean squares presented in these tables are based on the English measurement system.

TABLE 1. ANALYSIS OF VARIANCE FOR AVERAGE DAILY GAIN AND GAIN/FEED OF RATS IN EXPERIMENT A

		Mean squares				
		Average daily gain				
Source	df	Days 0 to 7	Days 8 to 14	Days 15 to 24	Days 0 to 24	Gain/ feed
Lysine	1	2.08	40.33	5.33	30.08	.01
Arginine (lysine)	4	60.21**	34.83	122.60	472.52*	.10
Within	42	13.52	19.71	54.66	162.71	.06
Total	47					

* P<.05.
** P<.01.

TABLE 2. ANALYSIS OF VARIANCE FOR AVERAGE DAILY FEED INTAKE OF RATS IN EXPERIMENT A

Source	df	Mean squares								
		Day 1	Day 2	Day 3	Days 4 to 5	Days 6 to 7	Days 8 to 14	Days 15 to 21	Days 22 to 24	Days 0 to 24
Lysine	1	.00	3.05	.15	2.52	6.90	14.30	95.77	6.16	.09
Arginine (lysine)	4	.64	2.10	.22	15.60*	15.80	668.57**	275.18	75.53*	3325.52**
Within	42	1.67	1.46	.85	4.75	9.39	97.64	125.68	22.75	804.35
Total	47									

* P<.05.
** P<.01.

TABLE 3. ANALYSIS OF VARIANCE FOR PLASMA AMINO ACID LEVELS (EXCLUDING CYSTINE) OF RATS IN EXPERIMENT A

Source	df	Mean squares										
		Argi-nine	Ly-sine	Orni-thine	Cit-rul-line	Methio-nine	Iso-leu-cine	Leu-cine	Va-line	Phenyl-ala-nine	Threo-nine	Tryp-to-phan
Lysine	1	1.03	5.36*	.13	.04	.00	.07	.09	.56*	.07	.01	.08
Arginine (lysine)	4	5.80**	2.52**	.26	.01	.06**	.06	.19**	.26	.05	.21	.43
Within	18	.32	.54	.09	.02	.01	.02	.04	.11	.02	.25	.47
Total	23											

* P<.05.
** P<.01.

TABLE 4. ANALYSIS OF VARIANCE FOR PLASMA CYSTINE LEVELS OF RATS IN EXPERIMENT A

Source	df	Mean squares Cystine
Lysine	1	2.59**
Arginine (lysine)	4	2.83**
Within	16	.03
Total	21	

** P<.01.

TABLE 5. ANALYSIS OF VARIANCE FOR ADJUSTMENT PERIOD GAIN, ADJUSTMENT PERIOD FEED INTAKE AND TEST DIET FEED INTAKE OF RATS IN EXPERIMENT B

		Mean squares		
Source	df	Adjustment period gain	Adjustment period feed intake	Test diet feed intake
Treatment	2	1.35	1.19	2.11
Within	12	34.56	104.79	.99
Total	14			

TABLE 6. ANALYSIS OF VARIANCE FOR PLASMA AMINO ACID LEVELS OF RATS IN EXPERIMENT B

		Mean squares										
Source	df	Argi-nine	Histi-dine	Ly-sine	Orni-thine	Methio-nine	Iso-leu-cine	Leu-cine	Va-line	Phenyl-ala-nine	Threo-nine	Tryp-to-phan
Treatment	2	33.80**	.20	.51	1.00**	.04	.03	.30	.47	.12	1.54	.02
Within	12	2.33	.08	3.63	.09	.08	.11	.50	.38	.18	1.92	.57
Total	14											

** P<.01.

TABLE 7. ANALYSIS OF VARIANCE FOR BODY WEIGHT LOSS AND AVERAGE DAILY FEED INTAKE OF RATS IN EXPERIMENT C

Source	df	Mean squares					
		Body wt loss	Average daily feed intake				
			Day 1	Day 2	Day 3	Day 4	Days 1 to 4
Lysine	1	58.78	.07	.84	3.74	.08	.00
Arginine (lysine)	4	59.53	26.30**	11.84	4.26	10.37*	146.20**
Within	30	54.44	3.31	4.58	4.68	3.46	29.72
Total	35						

* P<.05.
** P<.01.

TABLE 8. ANALYSIS OF VARIANCE FOR PLASMA LYSINE, ORNITHINE, CITRULLINE, ISOLEUCINE, LEUCINE, PHENYLALANINE, THREONINE AND TRYPTOPHAN LEVELS OF RATS IN EXPERIMENT C

		Mean squares							
Source	df	Lysine	Ornithine	Citrulline	Isoleucine	Leucine	Phenylalanine	Threonine	Tryptophan
Lysine	1	16.50**	.21	.00	.16	.15	.00	.05	.04
Arginine (lysine)	4	1.01	5.61**	.02	.06	.13	.06*	.07	.63
Within	10	.32	.14	.01	.02	.10	.01	.09	.23
Total	15								

* P<.05.
** P<.01.

TABLE 9. ANALYSIS OF VARIANCE FOR PLASMA VALINE LEVELS OF RATS IN EXPERIMENT C

Source	df	Mean squares Valine
Lysine	1	.17
Arginine (lysine)	4	.05
Within	8	.07
Total	13	

TABLE 10. ANALYSIS OF VARIANCE FOR PLASMA ARGININE AND METHIONINE LEVELS OF RATS IN EXPERIMENT C

Source	df	Mean squares Argi-nine	Methio-nine
Lysine	1	.10	.01
Arginine (lysine)	4	35.38**	.02
Within	9	.64	.01
Total	14		

** P<.01.

TABLE 11. ANALYSIS OF VARIANCE FOR AVERAGE DAILY GAIN OF PIGS IN EXPERIMENT 1

Source	df	Mean squares Average daily gain
Replication	3	.12
Lysine	1	.23**
Replication x lysine	3	.00
Arginine (lysine)	4	.02
Replication x arginine (lysine)	12	.02
Within	69	.02
Total	92	

** P<.01.

TABLE 12. ANALYSIS OF VARIANCE FOR AVERAGE DAILY FEED INTAKE AND GAIN/FEED OF PIGS IN EXPERIMENT 1

Source	df	Mean squares: Average daily feed intake	Mean squares: Gain/feed
Replication	3	.15	.00
Lysine	1	.01	.11**
Replication x lysine	3	.01	.02
Arginine (lysine)	4	.03	.01
Replication x arginine (lysine)	12	.02	.01
Total	23		

** P<.01.

TABLE 13. ANALYSIS OF VARIANCE FOR PLASMA AMINO ACID LEVELS OF PIGS IN EXPERIMENT 1

Source	df	Mean squares										
		Argi-nine	Histi-dine	Ly-sine	Orni-thine	Cit-rul-line	Me-thio-nine	Iso-leu-cine	Leu-cine	Va-line	Phenyl-ala-nine	Threo-nine
Replication	3	2.61	1.80	.95	1.21	.39	.05	.07	.22	.57	.22	.43
Lysine	1	.00	.78	43.17**	.09	.38**	.12*	.26	.04	1.15*	1.11*	39.76**
Replication x lysine	3	.45	.09	.49	.04	.01	.01	.03	.16	.08	.06	.29
Arginine (lysine)	4	3.50**	.40	.28	1.80**	.02	.02	.01	.06	.04	.05	.84*
Replication x arginine (lysine)	12	.36	.27	.23	.10	.05	.01	.03	.09	.13	.09	.23
Total	23											

* P<.05.
** P<.01.

TABLE 14. ANALYSIS OF VARIANCE FOR AVERAGE DAILY GAIN OF PIGS IN EXPERIMENT 2

Source	df	Mean squares Average daily gain
Replication	4	1617.81*
Treatment	3	72.05
Replication x treatment	12	316.30
Within	56	417.03
Total	75	

* P<.05.

TABLE 15. ANALYSIS OF VARIANCE FOR AVERAGE DAILY FEED INTAKE AND GAIN/FEED OF PIGS IN EXPERIMENT 2

Source	df	Mean squares Average daily feed intake	Gain/ feed
Replication	3	1678.80	200.00
Treatment	4	751.52	437.25**
Replication x treatment	12	404.60	24.67
Total	19		

** P<.01.

TABLE 16. ANALYSIS OF VARIANCE FOR PLASMA ARGININE, HISTIDINE, LYSINE, ORNITHINE, METHIONINE, ISOLEUCINE AND PHENYLALANINE LEVELS OF PIGS IN EXPERIMENT 2

Source	df	Mean squares						
		Argi-nine	Histi-dine	Ly-sine	Orni-thine	Methio-nine	Iso-leucine	Phenyl-alanine
Replication	4	2.41	.05	.42	.53	.00	.21	.17
Treatment	3	66.27**	1.17**	8.64*	14.21**	.29*	.40	2.68**
Replication x treatment	12	1.97	.11	1.99*	.22	.06	.24	.09
Within	20	1.05	.13	.62	.29	.04	.18	.08
Total	39							

* P<.05.
** P<.01.

TABLE 17. ANALYSIS OF VARIANCE FOR PLASMA THREONINE, VALINE, CYSTINE AND LEUCINE LEVELS OF PIGS IN EXPERIMENT 2

Source	df	Mean squares			
		Leu-cine	Va-line	Cys-tine	Threo-nine
Replication	4	.86	.17	.19	13.98**
Treatment	3	.43	5.43**	.18	32.46*
Replication x treatment	12	.57	.49	.08	8.05*
Within	19	.51	.80	.06	2.82
Total	38				

* P<.05.
** P<.01.

TABLE 18. ANALYSIS OF VARIANCE FOR PLASMA TRYPTOPHAN LEVELS OF PIGS IN EXPERIMENT 2

Source	df	Mean squares
		Tryp-tophan
Replication	4	1.09
Treatment	3	.37
Replication x treatment	12	1.00
Within	15	1.16
Total	34	

TABLE 19. ANALYSIS OF VARIANCE FOR PLASMA CITRULLINE LEVELS OF PIGS IN EXPERIMENT 2

Source	df	Mean squares Cit-rulline
Replication	4	.19**
Treatment	3	.10
Replication x treatment	12	.03
Within	16	.04
Total	35	

** P<.01.